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*joint proceedings*

# SECOND GENETICS WORKSHOP of the Society of American Foresters

and the

# SEVENTH LAKE STATES FOREST TREE IMPROVEMENT CONFERENCE

**OCTOBER 21 - 23, 1965**

NORTH CENTRAL FOREST EXPERIMENT STATION
C. R. King, Director
FOREST SERVICE
U. S. DEPARTMENT OF AGRICULTURE

# FOREWORD

From 1953 to 1963 the Biennial Proceedings of the Lake States Forest Tree Improvement Conferences have been published by the Lake States Forest Experiment Station. The 1965 meeting was unique in that it combined the Seventh Conference with the Second Genetics Workshop sponsored by the Committee on Forest Tree Improvement of the Society of American Foresters. Although the Workshop was national in scope, it provided the technical program for the Lake States Forest Tree Improvement Conference.

The North Central Forest Experiment Station is glad to publish these Joint Proceedings, thereby continuing the support formerly provided by the Lake States Forest Experiment Station.

D. B. King, Director
North Central Forest Experiment Station

## ACKNOWLEDGMENTS

When the SAF Committee on Forest Tree Improvement met at Denver, Colorado, in September 1964 under the chairmanship of R. Z. Callaham, it decided to hold a Forest Genetics Workshop in Michigan in October 1965, prior to the National SAF meeting scheduled for Detroit from October 24 to 28. Paul Rudolf informed the group that the Lake States Forest Tree Improvement Committee had already decided to hold the Seventh Lake States Forest Tree Improvement conference in Lower Michigan during the fall of 1965, and he suggested that this regional conference and the national workshop be combined. This suggestion was accepted both by the SAF Committee on Forest Tree Improvement and by the Lake States Forest Tree Improvement Committee.

Hans Nienstaedt was asked to plan the technical program for the SAF Committee on Forest Tree Improvement, and Jonathan Wright, Vice Chairman of the Lake States Forest Tree Improvement Committee, was asked to arrange for the meeting place and facilities, and for the field trip. Accordingly, Nienstaedt issued a call for papers through the Journal of Forestry and developed a program containing 22 papers. Wright made arrangements to hold the meeting at Michigan State University's Biological Station near Hickory Corners, Michigan, and, together with Walter Lemmien (Superintendent of the Michigan State University Kellogg Forest), outlined and conducted the very interesting and successful field trip.

It is a big job to plan, arrange, and conduct a workshop-conference such as this. On behalf of our Committees, we should like to thank all who helped in this task. By separate letter (copy later in this Proceedings) we have expressed our appreciation to Michigan State University for the fine facilities provided.

We also want to make special note of our thanks to Director King for publishing the Proceedings and to Mrs. L. P. Olsen of the North Central Forest Experiment Station for editing them.

Scott S. Pauley, Chairman
Lake States Forest Tree Improvement
Committee

Hans Nienstaedt, Chairman
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# VARIATION IN JUVENILE GROWTH AND WOOD PROPERTIES IN HALF-SIB COTTONWOOD FAMILIES

by R. E. Farmer, Jr., and J. R. Wilcox[1]

Maximum early gains in the genetic improvement of eastern cottonwood (*Populus deltoides* Bartr.) in the lower Mississippi Valley are most likely to be achieved through identification and commercial vegetative propagation of superior genotypes. A relatively simple improvement program having this goal might include evaluation of progeny from phenotypically superior mother-trees followed by clonal tests of outstanding juvenile phenotypes selected from progeny. Such an approach is being used in the first phase of the cottonwood improvement program at the Southern Forest Experiment Station. This paper describes a short-term one-parent progeny test conducted under nursery conditions to provide data on juvenile variation.

## Methods

Twenty-five 20- to 30-year-old female trees were chosen for their phenotypic superiority in size and form; all occurred in stands along the Mississippi River between Clarksdale and Vicksburg, Mississippi. Seed collected from the trees in June 1962 was sowed in 4-inch peat pots filled with a standard potting mixture (sand:peat:loam, 1:1:1). Resulting seedlings were grown for 1 month in greenhouses. In early August potted plants were transferred to a nursery site characterized by a Commerce silt loam soil. The 25 families were planted in a randomized block design with 10 replications of 18-tree row plots. Rows were 3 feet apart, plants were 2 feet apart within rows, and a 2-row border was planted around the test. Seedlings were irrigated immediately after they were planted and re-irrigated and cultivated as necessary during the balance of the summer.

Total height and diameter at 1.5 inches above the soil surface were recorded in December 1962. The seedlings were then clearcut at groundline, and a second season's growth on the rootstocks was observed. This cutting was necessary because the plants were spaced so closely and grew so fast that they might have become stagnated during the second year. After growth resumed in the spring, clumps of sprouts were thinned to one stem.

At the end of the second growing season, total height and diameter at 1 foot above the soil surface were measured on all trees. Specific gravity and fiber length were evaluated from wood samples taken at the base of the first six trees in each plot. Specific gravity was determined from the green volume and oven-dry weight. For fiber-length determinations, six 1-mm.-wide slivers were cut in matchstick-like form from the outer portion of each wood sample. The slivers were macerated in a 1:1 mixture of glacial acetic acid and 30-percent hydrogen peroxide at 50° C. for 36 hours. The macerated, bleached fibers were washed in distilled water, stained for 15 minutes in a Bismarck Brown solution (1 gm. stain in 100 ml. of 50% ETOH, filtered) and mounted in a 1.5-percent agar solution on glass slides. Sixty fibers per sample were measured with a modified ampliscope (Echols 1959; Wilcox *et al.* 1964) at 52 X, and the sample mean was used as the unit of analysis.

Specific gravity and fiber length of wood samples from parent trees were also determined by the above techniques. Two cores 11 mm. in diameter were taken from each tree. Specific gravities of whole cores were measured and fiber samples were taken in the last growth ring.

Data were analyzed as outlined in table 1. Because of unequal numbers of trees per plot, an approximation of the within-plot variance was computed from individual-tree data in every tenth plot, and the harmonic mean of plants per plot was used to convert this value to the appropriate scale for comparison with other mean squares. Narrow-sense heritability values were calculated, using the formula in table 1. Family means were compared through use of Harter's (1960) values for Duncan's new multiple range test.

## Results

Mean family height growth varied significantly (0.01 level) from 3.1 to 4.6 feet in 1962 and from 11.9 to 14.2 feet in 1963. Heights of individuals ranged from 0.8 to 6.8 feet in 1962 and from 4.6 to 21.1 feet in 1963.

[1]*Plant geneticists stationed respectively at the Southern Hardwoods Laboratory, Stoneville, Miss., and the Institute of Forest Genetics, Gulfport, Miss.; both the Laboratory and the Institute are part of the Southern Forest Experiment Station, Forest Service, U.S. Dept. of Agriculture. The Southern Hardwoods Laboratory is maintained in cooperation with the Mississippi Agricultural Experiment Station and the Southern Hardwood Forest Research Group.*

Table 1.--Analysis of variance and narrow-sense heritabilities for several characters in a cottonwood one-parent progeny test

| Item | | Height | | Diameter | | Specific gravity | Fiber Length | Estimated mean square |
|---|---|---|---|---|---|---|---|---|
| | | 1962 | 1963 | 1962 | 1963 | | | |
| Source of variation | df | | | | | | | |
| | | | | MEAN SQUARE | | | | |
| Replications (R) | 9 | 0.7456 | 92.8262 | 8.9700 | 4.572844 | 0.064489 | 0.083456 | |
| Families (F) | 24 (1) | 2.0517 | 3.3640 | 19.8058 | .300779 | .008279 | .023846 | $\sigma^2_W + K\sigma^2_{RF} + RK\sigma^2_F$ |
| Replications X Families | 1/ 215 (2) | .1233 | 1.2359 | 2.9407 | .116167 | .001437 | .005358 | $\sigma^2_W + K\sigma^2_{RF}$ |
| Within plot (W) | (3) | .0401 | .2794 | .5272 | .063520 | .000452 | .002287 | $\sigma^2_W$ |
| | | | | VARIANCE COMPONENT | | | | |
| $\sigma^2_F = (MS_1 - MS_2)/RK$ | | .0140 | .0130 | .1016 | .003077 | .000114 | .000308 | |
| $\sigma^2_{RF} = (MS_2 - MS_3)/K$ | | .0060 | .0583 | .1454 | .008774 | .000164 | .000512 | |
| $\sigma^2_W = MS_3$ | | .0401 | .2794 | .5272 | .063520 | .000452 | .002287 | |
| K= harmonic mean of plants per plot | | | | | | | | |
| | | | | HERITABILITY | | | | |
| $h^2 = \frac{4\sigma^2_F}{\sigma^2_F + \sigma^2_{RF} + \sigma^2_W}$ | | .93 | .35 | .52 | .16 | .62 | .40 | |

1/ Reduced by 1 because of 1 missing plot.

Diameter growth (mean for 1962, 0.5 in.; mean for 1963, 1.0 in.) followed the same pattern of broad variation as height, although familial differences were less and family rankings did not change greatly between the 2 years. Differences between replications were a major source of variation in both height and diameter growth, and reflect cottonwood's sensitivity to site differences within the 3-acre test area.

Family means for specific gravity ranged from 0.38 to 0.43 (mean = 0.40); density of individual trees varied from 0.31 to 0.52, a range similar to that observed in natural cottonwood stands of the lower Mississippi Valley. Mean fiber length for families ranged from 0.88 to 0.97 mm (mean = 0.92). The range in fiber length of individual trees (0.74-1.19 mm.) was close to that reported for mature *P. deltoides* (0.85-1.28 mm.) by Boyce and Kaeiser (1961), but greater than the range published by Kennedy and Smith (1959) for *P. trichocarpa* seedlings (0.42-0.60 mm.).

Narrow-sense heritabilities (table 1) for height decreased from 0.93 in 1962 to 0.35 in 1963; heritabilities for 1962 and 1963 diameter growth were 0.52 and 0.16. Specific gravity and fiber length had heritabilities of 0.62 and 0.40.

Narrow-sense heritability estimates were also calculated by regressing progeny mean values for specific gravity and fiber length on maternal parent values and doubling the regression coefficients; a value of 0.18 was obtained for specific gravity and of 0.32 for fiber length. These estimates, especially that for specific gravity, are considerably lower than those obtained by partitioning variance components. This result was not unexpected, since parent trees were growing on a variety of sites along the Mississippi River.

Genetic correlations in table 2, derived from covariance analyses indicate essentially no relationship between specific gravity and either diameter ($r_a = -0.07$) or fiber length ($r_a = 0.18$). There was, however, a strong positive genetic correlation between diameter and fiber length ($r_a = 0.80$). This relationship between growth rate and fiber length was observed in juvenile *P. trichocarpa* by Kennedy and Smith (1959).

Table 2.--Analysis of covariance and genetic correlations for diameter growth and wood properties in a cottonwood one-parent progeny test

| Item | | Diameter/ specific gravity | Diameter/ fiber length | Specific gravity/ fiber length |
|---|---|---|---|---|
| Source of variation | df | | | |
| | | | MEAN SQUARE | |
| Replications (R) | 9 | | -0.529854 | -0.242526 | 0.034092 |
| Families (F) | 24 | (1) | -.008219 | .048867 | .002998 |
| Replications X Families | 1/215 | (2) | -.005898 | +.000096 | .000956 |
| Within plot (W) | | (3) | -.000710 | +.002716 | .000161 |
| | | | VARIANCE COMPONENT | | |
| $\sigma^2_F = (MS_1 - MS_2)/RK$ | | | -.000039 | .000769 | .000034 |
| $\sigma^2_{RF} = (MS_2 - MS_3)/K$ | | | -.000865 | 2/ 0.0 | .000132 |
| $\sigma^2_W = MS_3$ | | | -.000710 | +.002716 | .000161 |
| | | | GENETIC CORRELATION | | |
| | | | -.0667 | .7994 | .1818 |

1/ Reduced by 1 because of 1 missing plot.

2/ Considered as 0.0 since within-plot variance exceeded replication X family variance.

## Discussion and Conclusions

The marked differences in heritabilities between the 2 years for height and diameter and between the two methods of calculation for the wood property data illustrate the limitations of the values. They are descriptive statistics for a particular experiment. Only as the experiment is typical of a species or population will the heritabilities be typical; therefore caution is needed in their interpretation and particularly in their extension to other populations.

The narrow-sense heritabilities for growth parameters in 1963 are in the general range reported for juvenile half-sib progeny tests by Matthews *et al.* (1960) and Wright (1963). The difference between height and diameter-growth heritability in 1962 and 1963 may be due to several causes. For one thing, environmental preconditioning in the greenhouse while plants were growing in family blocks may have accentuated 1962 family differences. Second, the difference in competition between 1962 and 1963 may have altered variation patterns. Cutting plants back to root stocks after one season's growth may also have had an effect.

Narrow-sense heritabilities for wood properties in cottonwood have not been reported. Our estimates of 0.62 for density and 0.40 for fiber length are within the range for juvenile conifers (Stonecypher *et al.* 1964; van Buijtenen 1962; Zobel 1961). They indicate opportunity for moderate genetic gain via family selection. Of perhaps greater significance is the strong genetic correlation between diameter and fiber length ($r_a = 0.80$), which indicates that selection for rapid growth will result in a concurrent improvement in fiber length. Other genetic correlations suggest little opportunity of increasing density by selecting for other characters.

Although a high degree of variation in growth is present in our material, lack of data on juvenile-mature correlations suggests the need for extensive selection in nursery tests. In a 10-year study of growth patterns in highly variable progenies

of hybrid poplar, Bialobok (1963) found that ranking of individual trees within progenies changed annually for 5 to 7 years. The poorest were easily identified after first-season growth, but within the better stems rearrangement was considerable. Thus, in our material, clonal testing of the top 10 percent of phenotypes in the top 20 percent of families would appear to be a safe and productive procedure at this stage of breeding.

Juvenile cottonwood's rapid growth and sensitivity to minor site differences (both demonstrated in this test) indicate that special care is necessary in the design and conduct of nursery progeny tests. Heterogeneity common to the alluvial soils in the lower Mississippi Valley is thus a major problem in testing. The most accurate results can perhaps be secured with intensive site preparation and culture (as for agricultural crops) and sensitive experimental designs such as the lattices. At least 4- by 4-foot spacing is desirable if material is to be observed for 2 years.

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# SELECTION FOR PRECOCIOUS FLOWERING IN PINUS SYLVESTRIS


**by Henry D. Gerhold**[1]


The reproductive systems of forest trees that have evolved in wild habitats are usually characterized by delayed flowering age, cyclic seed years, and possibly an optimum, rather than a maximum, number of seeds per tree. Presumably these characteristics are favored by natural selection, though not necessarily in all species or in all situations.

During the development of a cultivated variety from wild populations, man may drastically change the reproductive system of a species, and the environment in which it grows. The new forces in man's scheme of selection — controlled mating and artificial regeneration — will tend to multiply genes that favor seed production at an earlier age, at more regular intervals, and in larger amounts, unless counter measures are taken. This raises questions about the effectiveness of these forces in altering features of seed production, as well as the desirability of any change they may bring about.

Earlier and increased fruitfulness has some obvious potential advantages for genetic experiments, for breeding improved varieties, and for mass-producing improved seed (see Green & Porterfield 1962; Matthews 1963; Schrock 1949; Wright 1964). The advantages increase as rotations become shorter, as growth rates improve, and as juvenile selection becomes more feasible. They must be weighed against any adverse effects on yield or quality that accompany increased seed productivity.

This paper reports on flowering characteristics of young Scotch pines, and attempts to organize our present knowledge about precocious flowering in *Pinus sylvestris,* with reference to breeding improved Christmas tree varieties. In such a program, both selection and breeding can start by age 7. While this is not typical of tree breeding programs in general, it may serve as a useful example to give us some insight into a problem that we may face in the future with other species.


[1]*Assistant Professor, School of Forest Resources, Pennsylvania State University, University Park.*


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## Materials and Methods

Two categories of trees were studied. The largest group consists of 34 open-pollinated families, most of which are progenies of plantation or ornamental trees selected for Christmas tree properties. Many of the seed parents were less than 30 years old, and thus there may have been some mild, but not intentional, selection favoring precocious flowering. Unfortunately there is no provenance information for these trees, but it is likely that countries such as Scotland, France, Germany, and Poland may be represented. Two-year-old seedlings grown at Washington Crossing, N.J., were planted in 1959 in 30 randomized blocks at each of 3 locations in Pennsylvania (also at 3 locations in New Jersey and 3 in New York). They are now 8 years old from seed.

The second category consists of a commercial variety named "Nye Branch", which was included in the same experiment. The only difference in handling was that these seedlings were raised at the Nye Branch Nursery, Porter, Penn. The variety has been derived from a single plantation of unknown origin. In three subsequent generations seed has been collected from the numerous young trees that flowered in Christmas tree plantations, so that a type of mass selection for precocious flowering has been practiced inadvertently. The seedlings in the experiment probably came primarily from the second generation, and may have contained admixtures from the first and/or third generations, although this cannot be stated with complete certainty. The original plantation was crowded and its seed production was low. The first generation may have flowered less than the second or third generations (according to the recollections of the present nursery owner), and these flower abundantly while still very young; but there have been no definitive measurements of any increase in flowering. Currently reverse selection is being practiced, and this may in time yield some interesting comparisons.

In the three Pennsylvania progeny test plantations, every individual was inspected periodically for the presence of ovulate and staminate strobili. Quantitative estimates were made on June 9, 1965, at the University Park plantation.[2]

## Discussion of Results

Precocity of flowering may be measured in various ways, including age at which flowering begins, proportion of trees flowering in early years, and abundance of flowers on young trees. The numbers of trees that have flowered at three locations are shown in table 1. Very few flowered by age 4, after which there was a fairly steep increase

Table 1.--Number of Scotch pine trees flowering at 3 Pennsylvania locations (based on 1050 trees at each)

| Age from seed | Univ. Park | | Hallston | | Akeley | |
|---|---|---|---|---|---|---|
| | Female | Male | Female | Male | Female | Male |
| 3 | 2 | - | - | - | - | - |
| 4 | 3 | - | 51 | - | 17 | 1 |
| 5 | 41 | 0 | 212 | 1 | 48 | -0 |
| 6 | 60 | - | 144 | - | 166 | - |
| 7 | 377 | 13 | 645 | 35 | 230 | 17 |
| 8 | 790 | 186 | - | - | - | - |

in the proportion of trees with ovulate strobili. By age 8, 75 percent of the trees at University Park were flowering, but less than 20 percent produced pollen, and most of these yielded rather small amounts. This pattern is in general agreement with other reports in the literature (Mergen 1961; Righter 1939; Sarvas 1962; Schrock 1949; Wright 1964). However, native Scotch pine in Scandinavian countries is reported to flower only at more advanced ages (Johnsson 1964), and Schrock (1949) has made the same claim for East Prussia, attributing the precocious flowering of certain trees to hybridization with introductions from France. It should be noted that a cyclic pattern appears only in the Hallston data, and that a maximum has not been reached at any of the locations, especially not in male flowering.

In 9 of the 34 families at University Park, more than 90 percent of their members bore female strobili at age 8, but only 1 family had male strobili on more than half of the trees. In 3 families less than 40 percent of the trees had female strobili. Every Nye Branch tree bore ovulate strobili, and over 90 percent had staminate strobili.

Some differences associated with location are illustrated in table 2. The values reflect not only the tendency to flower at an early age, but also the consistency of flowering during a 4-year period. The number of trees with conelets at Hallston, where the trees were tallest, was over twice

Table 2.--Cumulative number of conelet-bearing trees in Scotch pine families during 4 years at 3 Pennsylvania locations (based on 30 trees per open-pollinated family or variety, 1961-1964)

| Family or variety | Location | | |
|---|---|---|---|
| | Univ. Park | Hallston | Akeley |
| Average, 34 families | 13 | 27 | 12 |
| Range, 34 families | 0-36 | 2-60 | 0-41 |
| Nye Branch variety | 54 | 76 | 61 |
| Family number: | | | |
| 24 | 6 | 5 | 11 |
| 11 | 0 | 30 | 12 |
| 17 | 10 | 34 | 1 |
| 31 | 17 | 20 | 6 |

[2] *Thomas S. Hiscott, Graduate Assistant in Forestry, assisted with this work.*

as great as at University Park or at Akeley. The Nye Branch variety had 3 to 5 times as many flowering trees as an average family, and 1¼ to 1½ times as many as the most prolific family. Of the 34 families, 23 clearly conformed to the average pattern, that is, they had a high value at Hallston and much smaller, roughly equal values at the other two locations. The four families in table 2 differed most strikingly from the average pattern, indicating considerable interaction between environmental factors associated with locations and flowering precocity measured in this way.

The number of ovulate strobili per flowering tree is also of interest. At University Park in 1965, individual trees bore from 1 to an estimated 270. Of 920 trees, 235 had none, 360 had 1 to 10, 283 had 11 to 100, and 42 trees had over 100 ovulate strobili, 11 of these being Nye Branch. The family averages (number per flowering tree) ranged from 3 to 62, with a grand average of 27, while the comparable value for Nye Branch was 97. Families that bore large numbers of strobili also tended to begin flowering at an early age, but this does not necessarily indicate pleiotrophy or linkage. The association could easily result from similar selection pressures.

Clearly, there is in this species considerable variation in precocity, consistency, and abundance of female flowering upon which selection may operate. The variation can have significant genetic, environmental, and interaction components, and these must be evaluated for any trees that are to be subjected to artificial selection favoring or discriminating against these traits. The experimental design should provide for testing over a period of years, and probably at several locations. The data collection procedure should take into account the initial steep rate of increase in flowering within a few years, and the fact that different families and individuals may pass through this stage at different times. Some of the differences may be rather temporary, and these may be important in some situations but not in others.

The magnitude of the response to selection for precocious flowering is as yet unknown. The extreme values of the Nye Branch variety suggest that it could be very large, for the selection in that case has not been very intensive. However, we have no reference point for comparative purposes. Nevertheless, the startling fact remains that a Scotch pine variety exists, and may have been created with man's help, in which 90 percent or more of the trees at each of three locations have produced unexpectedly large numbers of ovulate strobili by age 7. It is quite possible that similar precocious varieties can be found or created in other species which also do not normally flower at an early age.

Does precocious flowering have adverse effects on yield or quality? No simple answer is possible at the present time. In our material there was a positive, but not close, correlation between height and number of ovulate strobili. This probably does not mean that the more prolific trees are inherently faster growing, but merely that among young flowering trees, those with larger crowns are able to produce more flowers. They may also be too young to show any effect. Schrock (1949) has reported that there was no significant difference in height or diameter between early-flowering Scotch pines 18 to 24 feet high and the non-flowering trees in the same plantation. Among eucalypt species planted in Italy, some of which produced seed at 3 to 5 years of age and at age 10 are up to 60 feet tall, there did not seem to be any correlation between growth rate and age at which flowering began, according to E. Giordano (personal communication). Even if adverse effects on growth are found in the future, they will not necessarily be large or apply to all cases (e.g., see Stern 1963).

Precocious flowering can have undesirable effects on the quality of Scotch pine Christmas trees. Male strobili are formed at the expense of needle fascicles, and when there is a large crop, branches can look rather bare. The presence of cones may have some sales appeal, but when they open at night in a warm living room, the crackling noise can be disturbing to light sleepers. Although these disadvantages are real and not to be dismissed lightly, they probably can be circumvented with some ingenuity.

## Conclusion

In my opinion, efforts to exploit precocious flowering could be extremely rewarding, without too great a risk of detracting from yield or quality if reasonable safeguards are incorporated. A small number of such attempts certainly could be justified by producing precociously flowering varieties for use in basic research, for example in theoretical genetic studies requiring several generations, or investigations of the physiology of flowering. They would also have value for tree breeding applications, if deleterious side effects are found to be non-existent, or small enough to be tolerated. The time barrier is one of our chief problems in timber production, especially in tree breeding, and precocious flowering is one promising means of overcoming it.

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# FAMILY INDICES FOR SEED-ORCHARD SELECTION


**by Gene Namkoong[1]**


Many seed orchard programs include a stage at which clones or seedlings are culled on the basis of progeny tests. Though breeding systems which involve progeny testing differ in efficiency, they all provide information on an individual's breeding value through its own performance and that of parental or seedling relatives. A combined index on any one trait, using information from a set of relatives, is desirable. However, tree breeders appear to be planning only limited use of indices on data from relatives. In so doing, they may be foregoing a considerable advantage, for the increased gain by index selection might often be substantial.

If the phenotypic mean value of an individual is measured by its own performance and that of his relatives, and these values are listed as $x_1$, $x_2$, . . ., $x_n$, then a single total evaluation (y) of these phenotypic means can be used to measure the individual's genotypic value (g) where:

$$y = b_1x_1 + b_2x_2 + \ldots + b_nx_n = \sum_j b_jx_j \qquad (1)$$

and where $b_1$, $b_2$, . . ., $b_n$ are unknown coefficients which weight the various phenotypic means. The problem is to choose the $b_j$ values so that culling in the seed orchard (on the basis of (y)) will provide maximum gain in (g).

The equation for genetic gain by simple selection in a normally distributed population is:

$$\triangle G = i\,\sigma_y h^2 = i\,\sigma_y \frac{\mathrm{Cov}\,(g, y)}{\sigma_y^2} = i\,\frac{\mathrm{Cov}\,(g,y)}{\sigma_y} \qquad (2)$$

Where i = the selection intensity (Z/p)
Z = the ordinate of the normal distribution at the truncation point
p = the proportion selected
Cov (g, y) = the covariance of the individual's true genetic value (g) and the index measurement of that value (y)
$\sigma_y$ = the standard error of (y)

Since the value of (i), which depends on the number of genotypes selected, will usually be predetermined, only the righthand ratio of (2) is maximized. This is done by the appropriate selection of weights (b) which in turn requires maximizing the correlation between (g) and (y) or minimizing the error in estimating (g) by the value of (y) (Smith 1936). In a manner analogous to regression theory, least-squares equations can be derived from $\hat{g}_i = y_i$

$$g_i = y_i + e_q$$
$$= \sum_j b_jx_{ij} + e_q.$$

The least-squares solution for (b) is:

$$\underline{b} = (\sigma_{x_j x_{j'}})^{-1} (\sigma_{\underline{gx}_j})$$

Where b = the column vector of weights
$(\sigma_{x_j x_{j'}})$ = the phenotypic covariance matrix
$(\sigma_{\underline{gx}_j})$ = the column vector of covariances between (g) and the $x_j$'s

Indices of this type were developed by Hazel (1943) for use with information on several traits and on several relatives. If the assumptions are approximately fulfilled, the index method will always be at least as efficient, and usually more efficient, than the tandem method (Hazel and Lush 1942, Young 1961).


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## Clonal Seed Orchards

In clonal orchards each ortet's performance in the woods (as judged by its deviation from local averages or controls), together with the relative mean score of its progeny family, will usually be available. For brevity, ramet data are ignored. To maximize gain, the weighting coefficients are computed from:

$$\begin{bmatrix} b_1 \\ b_2 \end{bmatrix} = \begin{bmatrix} \sigma^2_{x_1} & \sigma_{x_1 x_2} \\ \sigma_{x_1 x_2} & \sigma^2_{x_2} \end{bmatrix}^{-1} \begin{bmatrix} \sigma_{gx_1} \\ \sigma_{gx_2} \end{bmatrix}$$

where, $b_1$ = weight given to wild tree performance
$b_2$ = weight given to progeny test performance
$\sigma^2_{x_1}$ = phenotypic variance among wild trees
$\sigma^2_{x_2}$ = phenotypic variance among progeny means
$\sigma_{x_1 x_2}$ = phenotypic covariance of wild tree with progeny test performance (i.e., $\frac{1}{2}\sigma^2_A{}'$)
$\sigma_{gx_1}$ = covariance of additive genetic value with ortet performance (i.e., $\sigma^2_A$)
$\sigma_{gx_2}$ = covariance of additive genetic value with progeny performance (i.e., $\frac{1}{2}\sigma^2_A{}'$)

Since environments generally will be uncorrelated, $\sigma_{x_1 x_2}$ will usually be equivalent to the genetic covariance between parent and progeny mean. The covariance of the clone's additive genetic value and parental (ortet) performance ($\sigma_{gx_1}$) will be the total additive genetic variance of the wild population, and the covariance of clonal value and relative progeny performance ($\sigma_{gx_2}$) generally will be one-half of the additive genetic variance in the population of the clones.

When selection in the woods has truncated the population, and this reduced population is brought into the progeny test, a reduction in additive variance will usually have occurred. The selected clonal genotypes are the same as they were in the wild, and no new genotypes are created and no genetic recombination effected between the initial and clonal selection stages. The reduced additive variance ($\sigma^2_A{}'$), in relation to the wild woods population's $\sigma^2_A$ is:

$$\sigma^2_A{}' = \sigma^2_A \left[ 1 - \frac{\sigma^2_A}{\sigma^2_{x_1}} i(i - t) \right]$$

where (t) is the first-stage selection truncation point on a standardized basis (Finney 1956). Though this reduction is often ignored, it may be sufficient to change the index coefficients significantly. Therefore, instances of high and low reductions will be examined.

As an example of a simple situation, assume the following values for a low intensity of wild tree selection and low heritability, and the highly effective reduction of phenotypic variance from 100 in the wild population to 16 in the population of progeny means:

$$\sigma^2_A{}' = \sigma^2_A, \qquad \sigma^2_{x_1} = 100, \qquad \sigma^2_{x_2} = 16$$

These values give a ratio of $b_1 : b_2$ as 0.3 : 1.0, and maximum selection gain on culling orchard clones will be obtained by evaluating the data with these weights. For example, if the wild-tree and progeny-test scores for two clones are 100 and 150 vs. 120 and 140, respectively, their total scores would be 180 vs. 176.

The joint effects of the size of genetic variance and the relative size of $\sigma^2_{x_1}$ to $\sigma^2_{x_2}$ can be more readily visualized if the ratio of $b_1 : b_2$ is written as:

$$2 - \frac{\frac{1}{2}\sigma^2_A}{\sigma^2_{x_2}} : \frac{\sigma^2_{x1}}{\sigma^2_{x_2}} - \frac{\sigma^2_A}{\sigma^2_{x_2}}$$

when we assume $\sigma^2_A{}' = \sigma^2_A$.

Highly effective progeny testing at low heritabilities produces high ratios of $\sigma^2_{x_1}/\sigma^2_{x_2}$ and a heavier weighting of $b_2$ relative to $b_1$. Figure 1 shows a family of curves based on different levels of $\sigma^2_A$ at a standardized $\sigma^2_{x_1} = 100$. Two cases are presented. In the left-hand chart, no selection in the woods is assumed and hence $\sigma^2_A{}' = \sigma^2_A$. In the other chart, a selection of 1 : 10,000 in the woods is assumed and hence:

$$\sigma^2_A{}' = \sigma^2_A \left[ 1 - \frac{\sigma^2_A}{\sigma^2_{x_1}} \right] (.95)$$

Relative weights may be estimated by picking the curve closest to expected genetic variance and finding the point on it closest to the expected ratio of

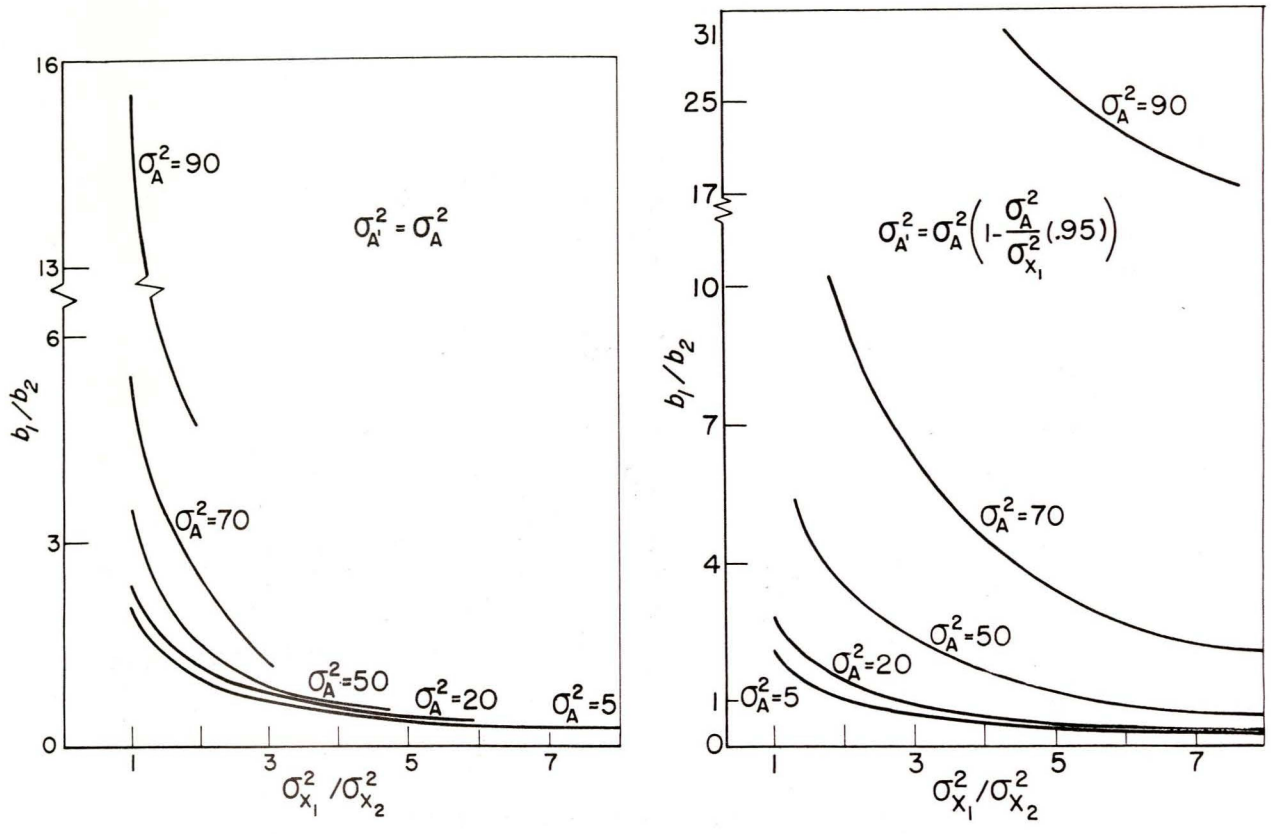


FIGURE 1. — Ratio of optimum weights for wild-tree data ($b_1$) and progeny mean data ($b_2$).

$\sigma_{x_1}^2/\sigma_{x_2}^2$. For example, at $\sigma_A^2 = 5$ and $\sigma_{x_1}^2/\sigma_{x_2}^2 = 5$, the ratio of $b_1/b_2$ is approximately 0.5. By interpolating between the curves for intermediate levels of $\sigma_A^2$ and "woods" selection intensities, one can estimate the relative weights to be assigned for a wide range of heritabilities $\sigma_A^2/100$, phenotypic variance ratios $\sigma_{x_1}^2/\sigma_{x_2}^2$, and selection intensities (i).

For higher $\sigma_A^2$ and lower $\sigma_{x_1}^2/\sigma_{x_2}^2$, the weight placed on progeny mean performance decreases.

Expected gains must be compared to evaluate index selection. The gain from selection based on progeny test data only is $i_2\ (1/2)\ \sigma_A^2{}'/\sigma_{x_2}$, where $i_2$ is the selection intensity of the orchard culling. The gain from index selection is:

$$i_2 \frac{\text{Cov (g, y)}}{\sigma_y}$$

where $\text{Cov (g, y)} = (b_1\ b_2) \begin{bmatrix} \sigma_{gx_1} \\ \sigma_{gx_2} \end{bmatrix}$

$$= (b_1\ b_2) \begin{bmatrix} b_1\sigma_{x_1}^2 + b_2\sigma_{x_1x_2} \\ b_1\sigma_{x_1x_2} + b_2\sigma_{x_2}^2 \end{bmatrix}$$

$$= (b_1\ b_2) \begin{bmatrix} \sigma_{x_1}^2 & \sigma_{x_1x_2} \\ \sigma_{x_1x_2} & \sigma_{x_2}^2 \end{bmatrix} \begin{bmatrix} b_1 \\ b_2 \end{bmatrix}$$

$$= \sigma_y^2$$

Thus, index selection gain is $i_2\ \sigma_y$.

The ratio $(\sigma_y) : (1/2\ \sigma_A^2{}'/\sigma_{x_2})$ is traced for several values of $\sigma_A^2$ and $\sigma_{x_1}^2/\sigma_{x_2}^2$ in Figure 2 assuming $\sigma_A^2 = \sigma_A^2{}'$, and alternatively assuming an initial selection of 1 : 10,000. The superiority of index selection is obvious. The effect of initial selection is to make index selection dramatically more advantageous, since genetic variance in the clonal

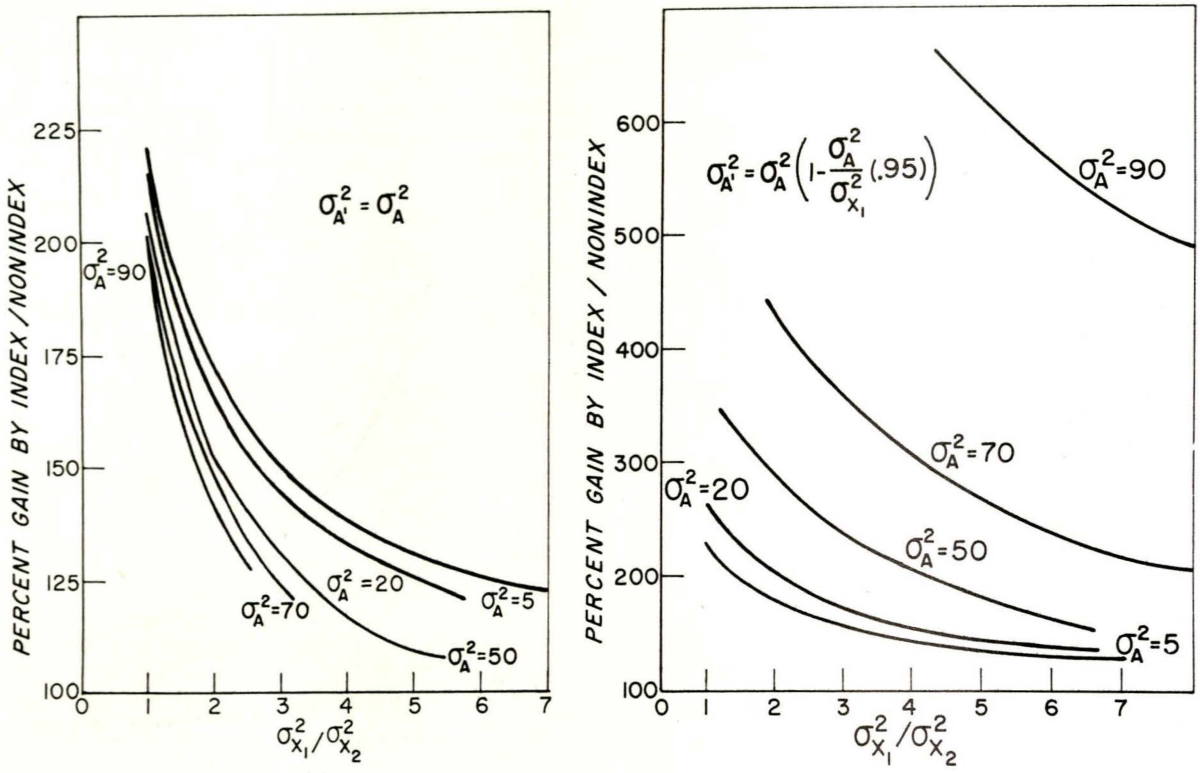


FIGURE 2. — Relative efficency of index selection over selection on progeny-test data only.

orchard is reduced. For example, under no initial selection and even at low $\sigma^2_A = 10$ and $\sigma^2_{x_1}/\sigma^2_{x_2} = 5$, index selection is 128 percent as effective in obtaining genetic gain as is selection only on the basis of progeny test means. For these same levels of $\sigma^2_A$ and $\sigma^2_{x_1}/\sigma^2_{x_2}$ but under the initial selection of 1 : 10,000, the superiority is 138 percent.

## Seedling Seed Orchards

For seedling seed orchards there are three sources of data on each potential orchard tree: the performance of the individual's wild maternal parent, its half-sib family, and its own relative performance in the orchard block. Although we must deal with three items of information, the principles remain the same. The problem is somewhat simplified by ignoring any reduction in genetic variance due to first-stage selection. If many genes at many frequencies are operating, the genetic recombination will regenerate genetic variance so that no increase or decrease is predictable for several generations of selection. It is also necessary to assume that at the time of final evaluation in the seedling orchard only one member, at most, of each half-sib family will be allowed to remain in each unit. The orchard may be divided into breeding blocks and each unit evaluated separately.

The weights may then be computed from:

$$\begin{bmatrix} b_1 \\ b_2 \\ b_3 \end{bmatrix} = \begin{bmatrix} \sigma^2_{x_1} & \sigma_{x_1x_2} & \sigma_{x_1x_3} \\ \sigma_{x_1x_2} & \sigma^2_{x_2} & \sigma_{x_2x_3} \\ \sigma_{x_1x_3} & \sigma_{x_2x_3} & \sigma^2_{x_3} \end{bmatrix}^{-1} \begin{bmatrix} \sigma_{gx_1} \\ \sigma_{gx_2} \\ \sigma_{gx_3} \end{bmatrix}$$

where $b_1$ = weight given to seed parent value
$b_2$ = weight given to half-sib family performance
$b_3$ = weight given to seedling's own performance
$\sigma^2_{x_1}$ = phenotypic variance of wild population
$\sigma^2_{x_2}$ = phenotypic variance of half-sib family means
$\sigma_{x_1x_2}$ = phenotypic covariance of half-sib family mean and seed parent value (i.e., $\frac{1}{2}\sigma^2_A$)
$\sigma^2_{x_3}$ = phenotypic variance among seedlings in an orchard block

$\sigma_{x_1x_3}$ = phenotypic covariance between wild tree and the individual seedling (i.e., $\frac{1}{2}\ \sigma^2_A$)

$\sigma_{x_2x_3}$ = phenotypic covariance of an individual seedling and its half-sib family (i.e., $\frac{1}{4}\ \sigma^2_A$)

$\sigma_{gx_1}$ = additive genetic covariance of the seedling with its maternal parent (i.e., $\frac{1}{2}\ \sigma^2_A$)

$\sigma_{gx_2}$ = additive genetic covariance of the seedling with its half-sib family mean (i.e., $\frac{1}{4}\ \sigma^2_A$)

$\sigma_{gx_3}$ = additive genetic covariance of the seedling with itself (i.e., $\sigma^2_A$)

Figure 3 shows families of curves similar to those of the previous case. Three levels of $\sigma^2_A$ and a range in phenotypic variance ratios are included for estimates of optimum weighting. Again the weights are highly dependent on the relative reduction of phenotypic variance from $\sigma^2_{x_1}$ to $\sigma^2_{x_2}$. If either $b_1$, $b_2$, or $b_3$ are set equal to 1, the other two relative weights can be found from the figures. It can also be seen that, as heritability increases and the ratio $(\sigma^2_{x_1} : \sigma^2_{x_2})$ approaches 1, the relative weight placed on family mean performance decreases very rapidly.

An evaluation of index selection can be made on the basis of an orchard selection of 1 : 10. In the tandem selection recommended by Goddard and Brown (1961) and Wright and Bull (1963), a selection would first be made on family means, and then on individuals within these families. Two cases may be arbitrarily chosen for comparison: one is the selection of 1 : 5 for family and 1 : 2 for individuals; the other is the selection of 1 : 3.16 for families and also 1 : 3.16 for individuals. Both schemes result in a final selection of 1 : 10. The ratios of gain by index vs. the former scheme are traced for several levels of genetic variance and testing effectiveness in figure 4. The ratios of gain by index vs. the latter 1 : 3.16 selections are similarly traced in figure 5. Again, the superiority of index selection is obvious.

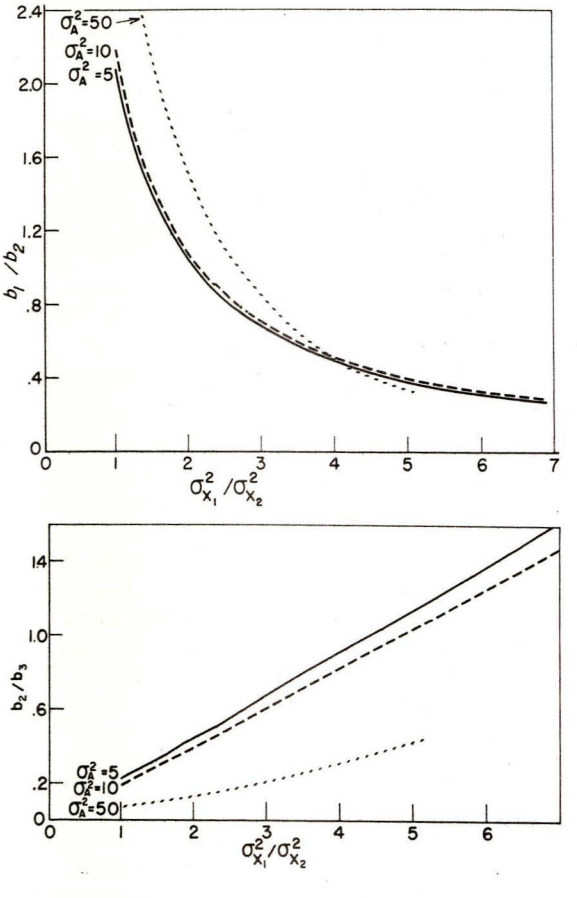


FIGURE 3. — Ratio of optimum weights to give to seed parent ($b_1$), half-sib family ($b_2$), and the seedling's own performance ($b_3$).

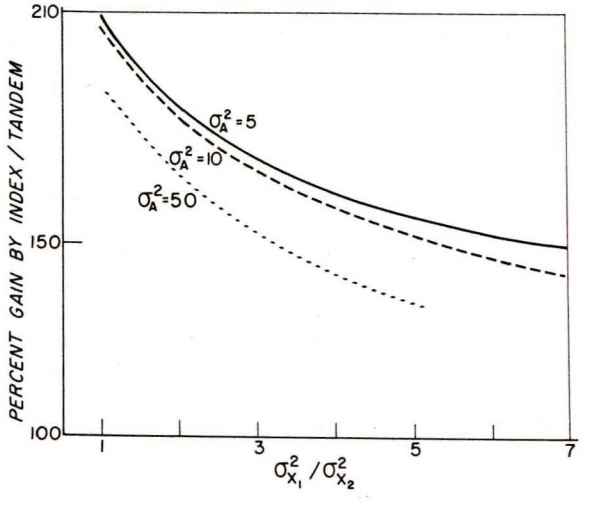


FIGURE 4. — Relative efficiency of seedling index selection over tandem selection at intensities of 1:5 for families and 1:2 for individuals.

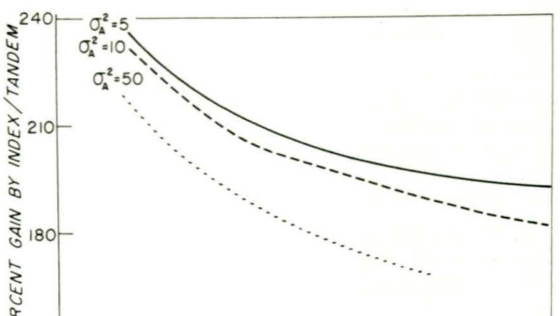


FIGURE 5. — Relative efficiency of seedling index selection over tandem selection at intensities of 1:3.16 for families and 1:3.16 for individuals.

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# LATTICE AND COMPACT FAMILY BLOCK DESIGNS IN FOREST GENETICS


**by E. Bayne Snyder**[1]


One of the principles of experimental design is that replicates be relatively homogeneous. Thus, in forest research a replicate is often assigned to a single crew for planting in a single day on a uniform site. When treatments are numerous, a large area is required per replication, and homogeneity of site is difficult to achieve. In this situation, crop scientists (LeClerg *et al.* 1962) frequently divide the replicate into sub-blocks. The most used of the incomplete block designs are the lattices. Another type of incomplete block designs, the compact family block (Hutchinson and Panse 1937; Federer 1955) — essentially a split-plot design with genetic rather than cultural whole plots — has also been advocated for certain genetic materials.

Such designs are frequently used in forestry abroad, e.g., Jeffers (1959), Langner (1961), and Schober (1961). This paper reports results from 16 current experiments in the United States. It also reviews literature dealing with specific designs and with "efficiency" as a means of comparing them. Many tree breeding programs are entering an era of comparing numerous genotypes, and it is hoped that this presentation will aid in choosing among designs.


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## "Efficiency" for Design Evaluation

Essentially, efficiency is the ratio, in percent, of the error variance of the randomized block design to the error variance of the design being compared. Various texts show how to calculate average effective error variances and efficiencies for lattice designs. In this presentation, it has been assumed that the within-block efficiency of the compact family block may be calculated as detailed by Federer (1955) for split plots.

Relative efficiency is often used to indicate how much saving in cost and land can be envisaged from a design. Thus, if an incomplete design had six replications and an efficiency of 150 percent, nine replications of a randomized block would be needed for equal accuracy. Day and Austin (1939) estimated that a 729-entry cubic lattice had an efficiency of 205 percent for ponderosa pine germination time. Beard (1954) calculated that, for wattle tree bark yield, a 10 by 10 lattice had an efficiency of 329 percent. Johnsson (1963) found a 5 by 6 rectangular lattice to have an efficiency of 133 percent for the heights of 10-year-old Scotch pine. Lester and Barr (1965), in a series of provenance tests for heights of 9- to 11-year-old red pine trees in rectangular lattices with replication sizes of approximately 1 to 2 acres, found that efficiencies varied according to planting site: 172, 179, 110, 105, 102, and 101 percent.

## Description of Designs

Limitation of this presentation to the two types of design does not imply that others are of no value. For example, the new augmented incomplete block designs (Federer 1961; Corsten 1962) should be useful where some treatments are to be more highly replicated than others. Even the discussion of the two designs is necessarily limited, so that full understanding of their principles and methodology will require additional study by the reader.

*Compact family blocks* — The compact family block is similar to a standard split-plot design in that comparisons among subplots are more accurate than those among whole plots. It is advantageous where genotypes divide themselves naturally into groups within which variation is smaller than between groups — say a provenance test where the progenies from 10 trees are collected per source and planted in a single sub-block (family block). Since there are no block adjustments as for lattices, comparison of entries in different sub-blocks is less precise than with a lattice. If there are sufficient whole-plot treatments, a lattice should be imposed on the whole plots.

The analysis, somewhat different from that for an ordinary split plot, is outlined by Panse and Sukhatme (1954). An equal number of subplots per family is not required, though it is desirable, and entries may be dropped from the experiment without complicating the analysis. Federer (1955) gives formulas for standard error. Cochran and Cox (1957) and Cockerham (1963) offer general discussion, and Johnsson (1952) reports a way of buffering against interfamily competition. John (1963) and Gates and Wilcox (1965) show how these designs may be used to obtain the genetic variance components of general and specific combining abilities and reciprocal effects.

*Lattices* — The array of lattices shown below allows for selection of a design over a wide range of treatments and replications. The size of the sub-block is designated as $k$. Thus, a 6 by 6 lattice has six sub-blocks per replication, each containing six entries. Another point shown is the increased replication required to achieve balance. Balance permits all comparisons at equal accuracy. When two designs are possible, the more balanced is preferred, but this is not to say that the investigator must adopt a balanced design when a partially balanced one fits his needs. Schutz and Cockerham (1962) suggest that an overall balanced design be used where the combination of locations and replications per location equals the required total number of replications for balance.

| Design | Minimum number of replications |
|---|---|
| **$k^2$ entries** | |
| Simple lattice (partially balanced) | 2 |
| Triple lattice (partially balanced) | 3 |
| Quadruple lattice (partially balanced) | 4 |
| . | . |
| . | . |
| . | . |
| Balanced lattice | $k + 1$ |
| **$k(k + 1)$ entries** | |
| Rectangular lattice (partially balanced) | 2 |
| . | . |
| . | . |
| . | . |
| Near balanced lattices | $k$ |
| **$k^3$ entries** | |
| Cubic lattice (partially balanced) | 3 |
| . | . |
| . | . |
| . | . |
| Balanced lattice | $k^2 + k + 1$ |

## Lattice Design Problems and Their Amelioration

Additional stratification within replicates necessitates more work and complications than are encountered for randomized complete block designs. The disadvantages must be weighed against the gains and minimized to the extent possible. Five alleged disadvantages are discussed below.

1. Lattice designs take more randomizations and bookkeeping at the planning stage than randomized blocks. First, to attain the proper number of entries for a design, the investigator may need to modify his original number. Then there is the chore of randomization. Clem and Federer (1950) supply random arrangements and Carmer (1965) furnishes a Fortran program for randomizations by an IBM 7094. Thompson (1958) shows how seed envelopes can be run through IBM accounting machines to be labeled. The advent of computers and automatic data processing equipment is thus minimizing the drudgery of planning and layout.

2. Wright (1962) claims that ". . . with sophisticated designs, it is necessary to search for the correct bundle to match the plot to be planted. This results in exposure of seedling roots to the air and increases variability." It is true that entries must be assigned and bundled by block within replication. This can be done at the time of packaging. Also, the field must be staked by block, but it is not necessary to number the blocks or the order of the plots within them, since both are at random. Thus, the bundle numbers that match

field plot numbers may be recorded after planting just as with a randomized complete block design. Depending on the experiment, I often prefer to keep track of plot assignments from the time of seed packeting.

3. According to Cochran and Cox (1957), hand calculations of the lattices may exceed those for randomized blocks by 20 to 150 percent. However, various developments ease the difficulty. Color coding of the III — or Z-blocks — in the treatment totals of some designs speeds up adjustment procedures. Staude (1963) supplies precalculated factors necessary for Duncan's Multiple Range Test. Carmer *et al.* (1963) supply automatic data processing methods for these designs. Homeyer *et al.* (1947) advocate that computers be used for 150 or more plots, or for a lesser total number if more than one character is being evaluated. In any event, the rapid development in computer technology has virtually eliminated the difference in calculation time.

4. The "insecurity" which condemns the lattices according to Evans *et al.* (1961) means, I assume, complications caused by missing plots. It is true that perennials grown in the rough are far more subject to losses than intensively cultivated annual crops. It is also true that missing-plot formulas are more complicated and hence more time-consuming for lattices than for randomized blocks.

However, these designs can be analyzed as randomized blocks and therefore are never appreciably less accurate than randomized blocks. The experimenter may choose whether to analyze them as randomized blocks or to complete the full analysis after data have been collected. Indeed, even without missing plots, the experimenter usually reverts to randomized block analysis if the efficiency is less than 105 or 110 percent. Cochran and Cox (1957) state that if there is any criterion for forming incomplete blocks, such a design is worth a trial in preference to a randomized block design.

I prefer to proceed with the full analysis in spite of missing plots. Missing-plot formulas are available in texts for most designs; Healy (1952) gives the procedure when an entire variety is missing. I have no information as to the percentage of missing plots that makes either randomized block or incomplete block analysis inadvisable. With experiments having few treatments, missing plots cause biases partly because of appreciable decreases in degrees of freedom, but where lattice designs are employed for 25 or more treatments, as is generally true, this effect is small. Healy and Westmacott (1956) and Yates (1960) have worked out computer analysis-of-variance methods for experiments with missing plots. The analyses of variances include lattices and are said to be suitable even if 10 percent or more of the plots are missing. I have a similar IBM 7094 program in Fortran for randomized blocks. In large experiments with missing values too numerous for hand calculations, my program will estimate missing-plot values for randomized blocks; these can be used in the lattice analysis (Goulden 1952). It is only a matter of time until improved computer programs are available.

5. The propriety of lattices for selection and studies of genetic variance components has been questioned. The subject was partially investigated by Schultz and Cockerhan (1962). They found that optimizing efficiency based on average error variances also optimizes efficiency based on expected gains. This was not true for some other types of incomplete block designs where additional computations are necessary to optimize gain. They also said that genotype-by-environment interactions caused by testing at different locations and years should not affect the superiority of any of the designs, since the interactions affect each of the expected gains in a similar fashion. Their study indicates that lattices are appropriate for selection studies or mean separation studies such as those for estimating combining abilities or testing provenances.

They further stated that gain from selection is not the only consideration, since simultaneous estimation of genetic variances is often desired, and that blocking may be desirable since it permits distribution of degrees of freedom more evenly among the mean squares as well as reduction in the error variances.

In further discussing genetic variances, Cockerham (1963) stated:

> Increase in land area generally increases the environmental variance because of soil heterogeneity which also reduces the reliability of components of variance per unit of land area. A solution . . . is . . . to use incomplete blocks. Other features, however, such as the distribution of degrees of freedom . . . are more pertinent to the reliability of the components of variance . . . of the joint mating-environment design. . . . One cannot accomplish anything by indiscriminately throwing the progenies into just any incomplete block environmental design which may fit. Care must be taken that the incomplete block design allows one to estimate the desired components of variance unconfounded with environmental components of the design.

Lester and Barr (1965) show that, for a series of provenance plantings, ordinary mean squares analysis is suitable. However, the breeder should consult a quantitative geneticist before he tests

structured genetic materials. Namkoong and Stern, while commending incomplete block designs for obtaining genetic components, have cautioned me that least squares computer analysis may be necessary to obtain "clean" components.

## Standard Controls as Alternatives to Incomplete Blocks

Evans *et al.* (1961) proposed a series of smaller randomized block tests with standard controls in preference to incomplete blocks. The design was used by Cech *et al.* (1963) in a study whose primary goal was to estimate components of variance. For varietal testing, however, Cochran and Cox (1957) and Schutz and Cockerham (1962) found that the control system is theoretically likely to be inferior in accuracy to a comparable incomplete design. Wishart and Sanders (1955), from results with crop plants, stated: ". . . but [the use of standard controls] has not proved to be a very satisfactory arrangement, even where it is arranged to have the same standard variety represented in all the experiments."

## Results from Experiments in the United States

The efficiencies of the 10 nursery or greenhouse experiments in table 1 vary from 100 to 274 percent for the lattices and from 92 to 163 percent for the compact family blocks. In general, the designs were not efficient for plants up to 3 weeks old and for characters such as *Melampsora* rust or fiber length. These limited results indicate that such characters are not affected by site heterogeneity. If they are the only characters studied, incomplete design would not be warranted. The designs were efficient for heights and weights of 1- or 2-year-old seedlings. In experiment 6, a chlorotic stunting occurred in part of one replication. The lattice design enabled use of all the data; otherwise, it would have been necessary to abandon this replication. If 110 percent is arbitrarily set as the efficiency required before a design is worthwhile, incomplete block designs were justifiable for one or more characters in 7 of the 10 nursery experiments.

The efficiencies of the seven field experiments in table 2 vary from 100 to 152 percent for the lattices and from 114 to 275 percent for the compact family blocks. Experiment 11, where the lattice was efficient, was on rolling land. Experiment 12, where efficiency was not attained, was with the same nursery stock but on a level crayfish flat. Two of the three experiments (11, 12, 13) showed no increased efficiency for fusiform rust data. The brown spot disease often infects fields irregularly for the first 2 years, and in experiment 15 the use of the lattice during this time was helpful. By the third year, inoculum had built up sufficiently to provide uniform infection, so that the lattice was ineffective.

Experiments 11 and 13 provide conflicting evidence as to whether incomplete blocks become more efficient as the trees grow older. In 11, efficiency for heights decreased from 138 percent at 3 years to 113 at 7 years, and in 13 it increased from 139 percent at 2 years to 275 at 5 years. It is suspected the two sites differ in the relative homogeneity of surface and subsurface conditions. Thus, in the field, incomplete blocks were valuable for one or more characters, i.e, had efficiencies of at least 110 percent in five of the seven experiments.

These results are confirmed by K. Stern (personal correspondence). He stated that more than 50 experiments at Schmalenbeck, West Germany, utilize incomplete block designs and that these designs are usually more efficient than randomized blocks.

## Summary

Testing numerous treatments often requires large replications on heterogeneous sites. Subdividing replications into smaller, more homogeneous incomplete blocks results in more precise comparisons. The compact family block (split-plot) is appropriate where genotypes divide themselves naturally into groups and where the within-group differences are smaller or of more interest than those between groups. Provenance studies with individual parents kept separate are examples. Lattices are appropriate when testing many genotypes whose differences are unknown or are of equal size or interest — for example, in the estimation of genetic gains, combining abilities, and components of variance.

Lattice layout and analyses may be speeded in various ways. Newly developed computer programs simplify and minimize the time of operations at all stages. Computers also minimize missing-plot problems, or the researcher can analyze lattices with missing plots as if they were randomized blocks. Therefore, whenever a large number of treatments necessitates large replication size, an incomplete block design is worth a trial.

Of 10 incomplete-block nursery or greenhouse experiments analyzed, 7 had efficiencies exceeding 110 percent in one or more characters, the range being 92 to 274 percent. The designs were efficient for heights and weights of 1- or 2-year-old seedlings. They were generally not efficient for nursery data up to 3 weeks, nor for such characters as fiber length or *Melampsora rust.* In the field, five of seven experiments were efficient, the range being 100 to 275 percent. In one experiment, efficiency for height increased during the period 2 to 5 years after planting; in another, it decreased during the interval 3 to 7 years.

Table 1.--Efficiencies of lattice and compact family block designs for Pinus species in the nursery or greenhouse

| Experiment number 1/ | Species Pinus- | Character | Age | Design | Number of- Blocks per replication | Number of- Replications | Number of- Entries | Efficiency (Percent) |
|---|---|---|---|---|---|---|---|---|
| 1 | elliottii | Germination percentage | 2 wk. | Rectangular lattice | 9 | 4 | 72 | 100 |
| | | Hypocotyl height | 3 wk. | do. | 9 | 4 | 72 | 103 |
| | | Seedcoat retention | 3 wk. | do. | 9 | 4 | 72 | 106 |
| | | Within-plot height variation | 1 yr. | do. | 9 | 4 | 72 | 106 |
| | | Weight | 1 yr. | do. | 9 | 4 | 72 | 116 |
| | | Height | 1 yr. | do. | 9 | 4 | 72 | 117 |
| 2 | elliottii | Seedcoat retention | 3 wk. | Compact family block | 8 | 3 | 23 | 92 |
| | | Within-plot height variation | 1 yr. | do. | 8 | 3 | 23 | 161 |
| | | Weight | 1 yr. | do. | 8 | 3 | 23 | 150 |
| | | Height | 1 yr. | do. | 8 | 3 | 23 | 148 |
| 3 | elliottii X echinata | Height | 1 yr. | Compact family block | 11 | 5 | 117 | 93 |
| 4 | palustris | Weight | 1 yr. | Rectangular lattice | 9 | 3 | 72 | 124 |
| | | Height | 1 yr. | do. | 9 | 3 | 72 | 159 |
| 5 | palustris | Germination percentage | 2 wk. | Simple lattice | 10 | 2 | 100 | 101 |
| | | Weight | 1 yr. | do. | 10 | 2 | 100 | 126 |
| 6 | palustris | Weight | 1 yr. | Simple lattice | 10 | 4 | 100 | 274 |
| 7 | deltoides | Height | 6 mo. | Triple lattice | 10 | 3 | 100 | 178 |
| | | Height | 1 yr. | do. | 10 | 3 | 100 | 198 |
| | | Diameter | 1 yr. | do. | 10 | 3 | 100 | 162 |
| | | Specific gravity | 1 yr. | do. | 10 | 3 | 100 | 117 |
| | | Fiber length | 1 yr. | do. | 10 | 3 | 100 | 104 |
| | | Melampsora rust | 1 yr. | do. | 10 | 3 | 100 | 100 |
| 8 | deltoides | Height | 6 mo. | Triple lattice | 10 | 3 | 100 | 108 |
| | | Height | 1 yr. | do. | 10 | 3 | 100 | 105 |
| | | Diameter | 1 yr. | do. | 10 | 3 | 100 | 104 |
| | | Specific gravity | 1 yr. | do. | 10 | 3 | 100 | 104 |
| | | Fiber length | 1 yr. | do. | 10 | 3 | 100 | 102 |
| | | Melampsora rust | 1 yr. | do. | 10 | 3 | 100 | 103 |
| 9 | ponderosa | Height | 2 yr. | Compact family block | 22 | 3 | 110 | 163 |
| 10 | menziesii | Photosynthetic efficiency | 1 yr. | Simple lattice | 10 | 2 | 100 | 102 |

1/ Experiments 1 through 8 are from nursery of Southern Forest Experiment Station, U.S. Forest Service. Data for experiments 7 and 8 were contributed by J. R. Wilcox and R. E. Farmer. They are from two closely spaced, short-term Populus clonal tests, one on a nursery site (7), and one on a forest site (8).

Data for experiment 9 are from Wells, O. O. Geographic variation in ponderosa pine (Pinus ponderosa Dougl. ex Laws). 1962. (Ph. D. thesis, Michigan State Univ., 112 pp.)

The Pseudotsuga of experiment 10 were grown in the greenhouse; data were contributed by R. K. Campbell, Weyerhaeuser Forestry Research Center, Centralia, Washington.

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Table 2.--Efficiencies of lattice and compact family block designs for Pinus species in field at Southern Forest Experiment Station

| Experiment number | Species Pinus- | Character | Years after outplanting | Design | Blocks per replication | Replications | | Entries | Efficiency |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | Number | Number | Acres | Number | Percent |
| 11 | elliottii | Survival | 1 | Triple lattice | 8 | 3 | 1.0 | 64 | 152 |
| | | Survival | 7 | do. | 8 | 3 | 1.0 | 64 | 109 |
| | | Height | 3 | do. | 8 | 3 | 1.0 | 64 | 138 |
| | | Height | 5 | do. | 8 | 3 | 1.0 | 64 | 129 |
| | | Height | 7 | do. | 8 | 3 | 1.0 | 64 | 113 |
| | | Fusiform rust | 7 | do. | 8 | 3 | 1.0 | 64 | 100 |
| 12 | elliottii | Survival | 7 | Triple lattice | 8 | 3 | 1.0 | 64 | 100 |
| | | Height | 3 | do. | 8 | 3 | 1.0 | 64 | 104 |
| | | Height | 5 | do. | 8 | 3 | 1.0 | 64 | 106 |
| | | Height | 7 | do. | 8 | 3 | 1.0 | 64 | 102 |
| | | Fusiform rust | 7 | do. | 8 | 3 | 1.0 | 64 | 101 |
| 13 | elliottii | Height | 2 | Compact family block | 8 | 3 | 1.0 | 23 | 139 |
| | | Height | 5 | do. | 8 | 3 | 1.0 | 23 | 275 |
| | | Fusiform rust | 5 | do. | 8 | 3 | 1.0 | 23 | 114 |
| 14 | palustris X taeda | Height | 5 | Compact family block | 7 | 6 | 1.0 | 25 | 105 |
| 15 | palustris | Brown spot | 2 | Simple lattice | 10 | 2 | 0.4 | 100 | 128 |
| | | | 3 | do. | 10 | 2 | 0.4 | 100 | 103 |
| 16 | palustris | Survival | 5 | Triple lattice | 10 | 6 | 1.4 | 100 | 110 |
| 17 | palustris | Height | 6 | Rectangular lattice | 9 | 3 | 0.6 | 72 | 114 |


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# SEGREGATION FOR CHLOROPHYLL DEFICIENCIES AND OTHER PHENODEVIANTS IN THE $X_1$ AND $X_2$ GENERATIONS OF IRRADIATED JACK PINE

by Thomas D. Rudolph[1]

Readily identifiable phenotypes, such as cotyledonary chlorophyll deficiencies, whose genetic basis can be established, would be highly useful in forest genetics research as genetic markers. In the genus *Pinus* mutants with severe deficiencies, such as albinos, will die shortly after seed germination while those with less severe deficiencies will not. In either case, however, the symptoms appear in early stages of growth and hence these types of mutants are especially useful as gene markers.

Where the mutation is lethal, the gene or genes are carried in natural populations in heterozygous combinations. Selfing or cross-pollination with a heterozygous carrier is necessary, therefore, to identify individual trees with a heterozygous genotype for this characteristic. Controlled self-pollination studies have shown that the pines are self-fertile in varying degrees (see review by Squillace and Kraus 1963), so identification of genotypes heterozygous for this character is possible. Scoring of the trait, however, must be done in the early seedling stages.

Chlorophyll deficiencies in seedlings of conifers have been reported by numerous investigators including Johnson (1945), Johnson (1948), Eiche (1955), Langner (1959), Cram (1960), Barnes *et al.* (1962), Squillace and Kraus (1963), Fowler (1962, 1965a, 1965c), and Snyder *et al.* (1966). They have found limited use as genetic markers in such studies as the determination of natural self-fertilization frequency (Cram 1960, Squillace and Kraus 1963, Fowler 1965a, 1965c) and in pollen dispersion studies (Langner 1952). Other applications in forest genetics research where genetic markers are essential could well utilize chlorophyll deficiencies alone or in combination with other easily recognized juvenile phenotypes.

To be of value, however, the genetic basis or mode of inheritance of such markers must be known. Results of a number of studies in conifers show an apparent 3:1 or single recessive gene ratio of normal vs. chlorophyll-deficient seedlings (Johnson 1945, Johnson 1948, Cram 1960, Barnes *et al* 1962). In these studies a limited number of parents were usually involved. In more recent studies, including a larger number of parent trees, the results show frequent deviations from the simple 3:1 ratio (Squillace and Kraus 1963, Fowler 1965a, 1965c, and Snyder *et al* 1966). These authors suggest several factors that may influence the observed segregation ratios. Most important appear to be environmental effects limiting deviant seedling emergence and possible pregermination lethals associated with the chlorophyll-deficient character. Certainly, larger populations than have been reported in the results of some studies must also be considered as essential for accurate definition of segregation ratios.

This report shows the frequency of chlorophyll deficiencies in a relatively large population of jack pine (*Pinus banksiana* Lamb.) in the second generation following X-irradiation of seed, and in a control population. Presented are segregation ratios based on open-, self-pollinated, and back-crossed progenies. Radiation effects, other than those concerned with phenodeviants, will be reported elsewhere.[2]

## Material and Methods

Jack pine seed collected in 1950 on the Chippewa National Forest in Minnesota was irradiated on March 14, 1951, with X-rays at dosages of 1,000 *r* and 4,000 *r*.[3] An unirradiated seedlot of the same origin was included as a control. The seed was sown in standard beds in the Hugo Sauer Nursery at Rhinelander, Wisconsin, on May 3, 1951. No seedlings survived after the first year in the nursery in the 4,000 *r* lot and only about one-fourth remained in the 1,000 *r* lot. Complete results of performance in the nursery will be reported elsewhere.[2]

In the spring of 1954, 200 seedlings from the control lot and 200 from the 1,000 *r* lot were planted on the Argonne Experimental Forest near Three Lakes, Wisconsin. Female strobili were evident beginning with the first year in the field, and the production of cones has steadily increased to the present.

During June and again in December 1961, 100 open-pollinated seeds were sown in sand in a greenhouse bench from: (1) 90 trees grown from

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[2]*Rudolph, T. D. Effects of X-irradiation of seed on $X_1$ and $X_2$ generations in* Pinus banksiana *Lambert. Ms. in preparation at Institute of Forest Genetics, Rhinelander, Wis.*

[3]*The seed used in this study was irradiated at Harvard University through the kind cooperation of Dr. Scott S. Pauley, at that time on the staff of the Maria Moors Cabot Foundation for Botanical Research at the University.*

the 1,000 *r* irradiated seed, (2) 45 subsamples from a bulk collection in the control plot, and (3) 45 subsamples from a bulk collection made in a natural stand in northern Wisconsin. Growing conditions in the greenhouse were the same as reported in another paper by Rudolph (1966) in these Proceedings.

Germination and early seedling development were observed periodically. The total number of deviant seedlings described and transplanted for further study from the two sowings were 324 from the individual trees grown from irradiated seed, 177 from the control plot collection, and 158 from the natural stand collection. From these preliminary trials, it was evident that we could further define the deviant types and possible differences in their frequencies due to X-irradiation of the seed from which the parents were grown only through study of both control-pollinated and open-pollinated individual tree progenies.

The controlled pollinations were made in 1962 and 1963. Ten isolation bags were employed on each of 24 trees in the 1,000 *r* plot in 1962, and on 86 trees in the 1,000 *r* plot and 21 trees in the control plot in 1963. The 24 trees included in the irradiated plot pollinated in 1962 were included in the 1963 pollination. The strobili in 8 bags on each tree were selfed, the strobili in 1 bag were pollinated with a mixture of Chippewa National Forest origin, and the strobili in 1 bag per tree were left unpollinated to serve as a control on the bagging technique. Pollination records and field labeling were maintained on an individual-bag and -branch basis to permit definition of possible radiation-induced chimeras on the basis of the seedling progenies.

Cones from the two years' pollinations were collected in September 1963 and 1964. The seed was extracted by dismembering each cone and was stored at 38°F. Beginning on March 18, 1965, the seed was sown in sand in a greenhouse bench. (For greenhouse conditions see second paper by Rudolph in these Proceedings.)

Germination counts were made after one month. Beginning at germination and during early seedling development, deviant seedlings were searched for frequently; and when the seedlings were 3 months old a final check was made. All unusual seedlings selected from the population, together with controls, were potted for further study.

## Results and Discussion

Among the most easily recognized phenodeviants in the early seedling stages of the material in this study were plants showing chlorophyll deficiencies in their cotyledons. Although quantitative differences in chlorophyll deficiency were evident between and, rarely, within progenies, it was felt that the differences could not be accurately classified as proposed by Gustafsson (1940). Furthermore, the expression of cotyledonary chlorophyll deficiencies is influenced by environmental conditions during seed germination and early seedling growth (Eiche 1955, Snyder *et al* 1966). All chlorophyll deficiencies were therefore considered as one type of deviant in the present study. Cross-breeding studies between individual trees heterozygous for the chlorophyll character are being continued, together with attempts to distinguish accurately between chlorophyll-deficient types among the pedigreed progenies.

Table 1 lists progenies showing more than 4 percent of the plants with this character. Chlorophyll deficiencies occurred at lower frequencies in 14 other selfed progenies. Of the 35 segregating progenies listed, 12, or more than one-third, showed an apparent segregation ratio of 15 normal: 1 mutant suggesting, in the case of the selfed progenies, heterozygosity for two recessive genes in the parent. Four of the 35 progenies segregated in a 3:1 ratio for chlorophyll deficiency in the cotyledons, indicating heterozygosity for one recessive gene in the parent. Nine others showed an apparent 7:1 ratio which does not result upon selfing either of the suggested genotypes. A 7:1 ratio could result from crossing a double heterozygote and a single heterozygote in a 2-gene model. This would be evident only in the backcrosses listed in table 1. However, only 2 of the 8 backcrosses listed show an apparent 7:1 segregation ratio.

Two other explanations are plausible. Perhaps the 7:1 ratio is a distorted 3:1 ratio resulting from a partial pre-germination lethal associated with the chlorophyll deficiency. On the other hand, the possibility cannot be discounted that the 7:1 ratio may actually result from an original 15:1 ratio distorted due to lethality independent of the chlorophyll deficiency, or in fact associated with the chlorophyll-sufficient character. This same type of phenomenon would explain the occurrence of 2:1 ratios in five of the progenies as being originally 3:1 ratios with a lethal associated with the chlorophyll character.

Some of the apparent 7:1, 2:1, and 1:1 ratios listed in table 1 also fit the simple 15:1 or 3:1 ratios, but with a low probability, in the chi-square goodness-of-fit test. This again suggests pre-germination lethals linked with the chlorophyll-deficiency factor in some cases and independent of it in others.

The presence of a relatively high proportion of chlorophyll-deficient seedlings in three of the open-pollinated progenies (N2, S42, and P43) suggests, on the basis of segregation ratios in their selfed progenies, that from 17 to 25 percent natural selfing occurred on these trees in the open-pollinated cones. These estimates agree quite well with

Table 1.--Segregation for cotyledonary chlorophyll deficiencies in open-pollinated, selfed, and back crossed[1/] jack pine individual tree $X_2$ progenies

| Tree number[2/] | Total no. of seedlings | No. of chlorophyll-deficient seedlings | Apparent ratio | P for chi-square goodness-of-fit test[3/] | Actual ratio |
|---|---|---|---|---|---|
| N2 Open-pollinated | 235 | 12 | -- | -- | 18.58:1 |
| N2 Self-pollinated | 190 | 59 | 2:1 | .60 | 2.22:1 |
| N2 Backcross | 34 | 6 | 7:1 | .40 | 4.67:1 |
| N8 Self-pollinated | 11 | 1 | 15:1 | .70 | 10.00:1 |
| N12 Self-pollinated | 31 | 5 | 7:1 | .55 | 5.20:1 |
| N15 Self-pollinated | 219 | 11 | 15:1 | .45 | 18.91:1 |
| N35 Self-pollinated | 19 | 1 | 15:1 | .85 | 18.00:1 |
| N35 Backcross | 23 | 1 | 15:1 | .70 | 22.00:1 |
| N41 Self-pollinated | 24 | 6 | 3:1 | 1.00 | 3.00:1 |
| N46 Backcross[4/] | 21 | 1 | 15:1 | .80 | 20.00:1 |
| N53 Self-pollinated | 60 | 11 | 3:1 | .20 | 4.45:1 |
| N53 Backcross | 29 | 2 | 15:1 | .80 | 13.50:1 |
| N62 Self-pollinated | 65 | 5 | 15:1 | .65 | 12.00:1 |
| N81 Backcross[4/] | 19 | 2 | 7:1 | .80 | 8.50:1 |
| N82 Self-pollinated | 294 | 32 | 7:1 | .40 | 8.19:1 |
| N82 Backcross | 159 | 8 | 15:1 | .50 | 18.87:1 |
| N90 Self-pollinated | 20 | 1 | 15:1 | .80 | 19.00:1 |
| S16 Self-pollinated | 8 | 3 | 2:1 | .80 | 1.67:1 |
| S18 Backcross[4/] | 107 | 1 | -- | -- | -- |
| S27 Self-pollinated | 15 | 1 | 15:1 | .95 | 14.00:1 |
| S42 Open-pollinated | 235 | 17 | -- | -- | 12.82:1 |
| S42 Self-pollinated | 53 | 15 | 3:1 | .60 | 2.53:1 |
| S42 Backcross | 40 | 9 | 3:1 | .70 | 3.44:1 |
| S47 Self-pollinated | 46 | 2 | 15:1 | .60 | 22.00:1 |
| S48 Self-pollinated | 37 | 11 | 2:1 | .65 | 2.36:1 |
| S66 Self-pollinated | 14 | 8 | 1:1 | .60 | 0.75:1 |
| S89 Self-pollinated | 14 | 2 | 7:1 | .85 | 6.00:1 |
| S100 Self-pollinated | 8 | 1 | 7:1 | 1.00 | 7.00:1 |
| P14 Self-pollinated | 141 | 18 | 7:1 | .90 | 6.83:1 |
| P43 Open-pollinated | 103 | 7 | -- | -- | 13.71:1 |
| P43 Self-pollinated | 89 | 30 | 2:1 | .80 | 1.97:1 |
| P51 Self-pollinated | 180 | 66 | 2:1 | .35 | 1.73:1 |
| P87 Self-pollinated | 85 | 9 | 7:1 | .60 | 8.44:1 |
| P88 Self-pollinated | 244 | 12 | 15:1 | .40 | 19.33:1 |
| P92 Self-pollinated | 112 | 10 | 7:1 | .25 | 10.20:1 |

1/ Seedlings resulting from open-pollinated seed were backcrossed with the female parent.

2/ N and S trees were grown from 1,000 r X-rayed seed. P trees are from non-irradiated control seed.

3/ P values rounded off to nearest 0.05.

4/ Trees N46, N81, and S18 had no chlorophyll-deficient seedlings in the open-pollinated or selfed progenies.

the frequency of natural selfing reported by Fowler (1965a). Nevertheless, the estimates may be somewhat high for two reasons. First, they do not take into account chlorophyll deficiencies that may have resulted from crossing with other heterozygous carriers. However, Snyder *et al* (1966) have found evidence from intercrossing 18 carriers that only 2 possessed a common gene for chlorophyll

deficiency, which suggests that the same phenotypes are produced by several non-alleles. Thus, the estimates of natural selfing presented here probably would not be greatly reduced by taking into account crossing among carriers. Second, since two of the three progenies under examination (N2 and P43) show a 2:1 segregation in their selfed progenies, possibly a lethal associated with the dominant chlorophyll factor is also tending to increase the proportion of chlorophyll deficiencies appearing in the open-pollinated progenies; if so, it would increase the apparent frequency of natural selfing.

The appearance of chlorophyll deficiencies in seedling progeny from backcrosses to female parents not showing chlorophyll deficiencies in their own selfed progeny (N46, N81, and S18) adds further to the uncertainty of the genetic basis for the chlorophyll character. From the response of the backcross progeny these female parents must be carriers of the recessive gene or genes for chlorophyll, but none of the homozygotes appear in the selfed progenies. Apparently all homozygotes for chlorophyll deficiency will die prior to germination when present in a largely homozygous genotype, but at least a few are viable in a more heterozygous genetic background. The possibility of cytoplasmic inheritance also cannot be excluded, and undoubtedly other explanations of this behavior could be offered. Indeed, possibly none of the explanations offered above could fully explain the extremely rare occurrence of chlorophyll deficiencies in the 14 selfed progenies not included in table 1.

No apparent differences in the frequency of trees carrying recessives for chlorophyll deficiencies were noted between the trees grown from irradiated seed and those from the control plot. In both groups, approximately one-fourth of the trees were found to be carriers of the recessive genes segregating in the ratios shown in table 1. This suggests that 1,000 *r* of ionizing radiation applied to seed did not increase the frequency of chlorophyll deficiencies in the second generation. However, since the trees from irradiated seed that were studied represent only a small part of the original population as a result of radiation-related mortality, it is also possible that the trees observed producing progenies segregating for this character represent only a small portion of those initially present. The high frequency of the recessive genes in this population does suggest a relatively high spontaneous mutation rate to the recessive condition at several loci. Squillace and Kraus (1963) present results which suggest a similarly high mutation rate for chlorophyll deficiency in *Pinus elliottii* var. *elliottii*. These authors also suggest that the frequency of the recessive genes may be influenced by other factors such as selection favoring heterozygotes over both homozygotes. Further study is needed to define the mutation rate or other factors responsible for the apparent high frequency of the recessive chlorophyll character.

Numerous other phenodeviants were found among the seedling progenies grown. Some of those readily recognized and occurring in significant frequencies are listed in table 2. On the basis of their frequencies in open-pollinated progenies and segregation upon selfing, a frequency of about 10 percent natural selfing is indicated. Of special interest are the chlorophyll-deficient seedlings that appear to regain a normal green color following the cotyledon stage. Among other things, this suggests that some mature trees may in fact be homozygous recessive for the chlorophyll characteristics manifested at the cotyledon stage. Fowler (1965b) has reported on a *Pinus resinosa* tree that may have this genotype. The reverse situation found in three progenies in which the cotyledons are green and the primary and secondary leaves initially deficient in chlorophyll presents another interesting phenomenon. This characteristic may be similar to the virescent type in *Pinus elliottii* reported by Kraus and Squillace (1964). Both types of seedlings survive at the end of 6 months in the greenhouse, but under natural conditions survival might be greatly reduced.

Two types of deviants which the author has not previously observed in large, non-irradiated populations and which may be radiation-induced mutants were found in Trees S30 and S91 (table 2). Both are apparently distinct mutants and are easily recognized. In the S30 progeny the mutants can be distinguished as soon as the seedlings germinate. Those in the S91 progeny become readily apparent with the development of primary leaves. These and other mutants isolated will be grown for further study.

Although no definite chimeras on the $X_1$ trees were identified from the $X_2$ progenies, several were suggested by differences in progenies from different parts of the individual X trees. Larger control-pollinated progenies will be grown from parts of the trees showing these apparent differences to more accurately define the type and extent of the chimeras if present.

## Summary and Conclusions

Approximately one-fourth of the trees both in the population grown from irradiated seed and in a control population were found to be carriers of genes recessive for chlorophyll deficiencies. Although this suggests that 1,000 *r* of ionizing radiation applied to seed had no effect on the frequency of chlorophyll deficiencies in the second generation, high early mortality in the irradiated population may obscure possible differences. Several segregating ratios for this characteristic were found. Possible explanations for the deviations from normal Mendelian ratios are presented. On

Table 2.--Segregation for deviant characteristics other than cotyledonary chlorophyll deficiencies in open- and self-pollinated jack pine individual tree $X_2$ progenies

| Tree no. | Type of pollination | Description of mutant | No. of seedlings | Ratio normal: mutant | Probable genotype of parent |
|---|---|---|---|---|---|
| N40 | selfed | Cotyledons normal green; primary leaves yellow | 52 | 3.0:1 | Heterozygous for one recessive gene |
| N53 | selfed | do. | 60 | 14.0:1 | Heterozygous for two recessive genes |
| N62 | selfed | do. | 65 | 64.0:1 | Heterozygous for three recessive genes |
| N12 | selfed | Cotyledons pale yellow; primary leaves green | 31 | 9.3:1) | Unknown; possibly heterozygous for one recessive gene with associated lethal. Apparent 9.1% natural selfing |
| N12 | O.P. | do. | 115 | 114.0:1) | |
| N82 | selfed | do. | 294 | 72.5:1 | Heterozygous for three recessive genes |
| S66 | selfed | do. | 14 | 2.5:1 | Heterozygous for one recessive gene |
| S30 | selfed | Thick, twisted, light-green hypocotyl; twisted thick cotyledons | 131 | 5.6:1) | Heterozygous for one recessive gene with associated lethal. Apparent 12.3% natural selfing |
| S30 | O.P. | do. | 272 | 53.1:1) | |
| S47 | selfed | Bright, yellow-green hypocotyl | 46 | 8.2:1 | Heterozygous for one recessive gene with associated lethal |
| S91 | selfed | Primary leaves very short, thick, and bluish-green. Epicotyl dwarfed | 357 | 5.6:1 | Heterozygous for one recessive gene with associated lethal |

the basis of the frequency of chlorophyll deficiencies in open-pollinated progenies as compared to ratios in selfed progenies, the proportion of natural selfing was found to be between 17 and 25 in the trees studied. Computed on the basis of other mutants, about 10 percent natural selfing was indicated. This percentage may be reduced by considering possible cross-pollination with heterozygous carriers of the same alleles.

In addition to the chlorophyll deficiencies, numerous other phenodeviants were noted and described. Segregation ratios and suggested genotypes ranging from one to three pairs of genes are presented. However, it is clear from the results that the mode of inheritance of potential genetic markers, such as chlorophyll deficiencies in the cotyledon stage, is not as easily definable as was previously believed on the basis of results with one or a few progenies.

The occurrence of two types of mutants for hypocotyl, cotyledon, and primary leaf characters

possibly induced by the X-irradiation of seed is described.

The presence of chimeras in the $X_1$ trees, although not definitely established, is suggested in several cases by differences between progenies from different parts of the same tree. Larger progenies than available in the present study are necessary to define possible radiation-induced chimeras.

Studies of the genetic basis for the phenodeviants observed in these populations are being continued.

From the results presented in this study it is apparent that much information on the genetic constitution of individual trees and populations may be gained by studying even limited numbers of progeny from self-pollinations. However, to accurately define the genetic behavior in successive generations and the possible influence of seed X-irradiation on this behavior, extremely detailed study of large progenies is required.

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# MONOTERPENE CONCENTRATION IN DOUGLAS-FIR IN RELATION TO GEOGRAPHIC LOCATION AND RESISTANCE TO ATTACK BY THE DOUGLAS-FIR BEETLE


**by J. W. Hanover and M. M. Furniss[1]**


The concentration of monoterpenes in *Pinus monticola* Dougl. has been shown to be genetically controlled (Hanover, in preparation). Genetic control of terpene concentration has been implied, also, from analyses of parents or interspecies hybrids in other species (Bannister et al. 1959; Williams and Bannister 1962; Smith 1964, and Forde 1964). Evidence that genes regulate the formation of plant terpenes has stimulated the use of the terpenes as an aid in population studies. Bannister et al. (1962) showed significant variation in $\alpha$-pinene and $\beta$-pinene between three populations of *Pinus radiata* in California. Also, Forde and Blight (1964) found that *Pinus muricata* Don in California could be classified into three different groups according to chemical composition. The monoterpene composition of *Pseudotsuga* has not been defined adequately although Cvrkal and Janak (1959) included *Pseudotsuga douglasii* Lindl. among several conifers they examined.

We were particularly interested in measuring the monoterpenes in wood oleoresin of *Pseudotsuga menziesii* var. *glauca* (Beissn.) Franco to determine the variation in monoterpenes (1) between individual trees, and (2) between groups of geographically separated trees. We also wished to


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determine the relation between monoterpenes and resistance to attack by the bark beetle, *Dendroctonus pseudotsugae* Hopk. Finally we studied seasonal variation of monoterpenes, viscosity of resin, and the relation between monoterpenes and several tree characteristics.

## Materials and Methods

Monoterpenes were sampled in 1964 at three localities in Idaho and Montana. The three localities (fig. 1) are sufficiently isolated to permit some genetic divergence between populations in each area. Oleoresin was sampled from groups of trees that survived attack by the bark beetles during 1962 and also from unattacked trees within Salmon River (A, Valley County) and Flathead Lake (B, Lake County) locations. At Moscow Mountain (C, Latah County) only unattacked trees were sampled. The 94 sample trees ranged in age from 81 to 184 years; diameter at breast height ranged from 24.9 to 76.7 cm; the last 10-year radial growth ranged from 1.4 to 41.6 mm; and crown class ranged from suppressed to dominant. Tree measurements did not appear to differ significantly between localities.

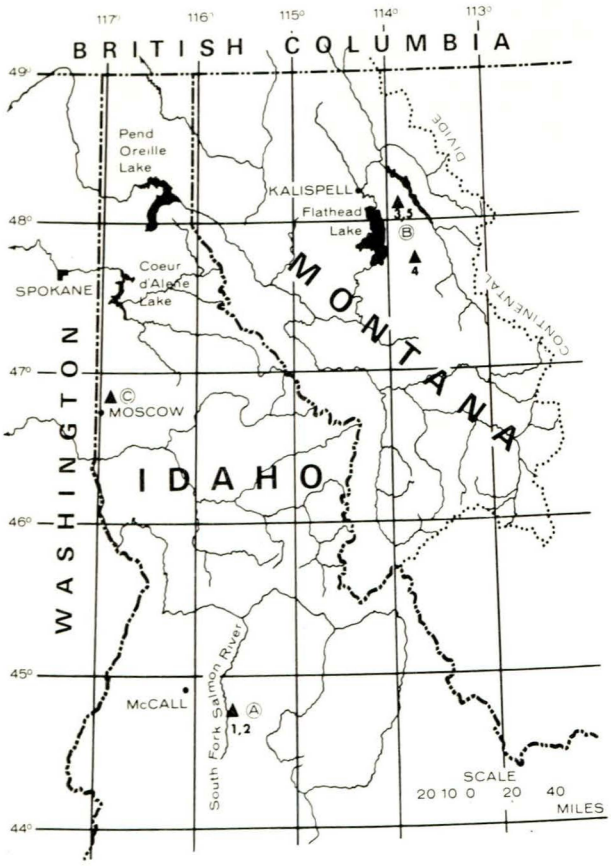


FIGURE 1. — Location of the three geographic areas and groups of trees within areas from which samples of Douglas-fir oleoresin were obtained. The areas are: (A) Salmon River in Valley County, Idaho; (B) Flathead Lake in Lake County, Montana; and (C) Moscow Mountain in Latah County, Idaho.

Differences in monoterpenes between attacked (resistant) and unattacked trees were tested by statistical analysis. When no significant differences occurred between groups, both types of trees within each area were combined for tests of population differences.

Oleoresin samples were collected during the latter part of the bark beetle flight period, between June 6 and 19, 1964. Fifteen trees of the Flathead Lake population were sampled again on October 14, 1964, to determine seasonal variation in their monoterpenes. Samples were collected and analyzed as follows: One day prior to collection of oleoresin, each tree was chopped to expose a cross-sectional portion of the wood. A 30-microliter sample of oleoresin that exuded from the wood resin ducts was drawn into a calibrated glass capillary tube. The tube of oleoresin was kept in a sealed centrifuge tube under refrigeration until analyzed soon afterwards.

Monoterpenes were analyzed qualitatively and quantitatively by gas-liquid chromatography. The oleoresin sample was dissolved in 50 microliters of acetone and a 2-microliter aliquot injected into an F&M Model 500[2] gas chromatograph with a flame ionization detector. The chromatograph column was ¼″ X 6′ stainless steel packed with 10-percent polypropylene glycol on 60-80 mesh Diatoport W-AW. Temperatures were: injection port, 195 C; column, 95 C; detector, 185 C. Helium flow rate was 165 ml per minute at the injection port.

Monoterpenes were tentatively identified by comparing relative retention times of the unknowns with those of known compounds. Identity was also determined by the addition of known monoterpenes to the plant samples to enhance the corresponding peaks. This procedure utilized both the polar polypropylene glycol column and a nonpolar Apiezon-L column packing. Agreement between two such columns is considered sound qualitative determination. With Apiezon-L, temperatures were: injection port, 195 C; column, 135 C; detector, 195 C. Helium flow rate was 35 ml per minute. Monoterpene concentration was expressed as a percent of total oleoresin by use of standard curves relating peak area (disc integrator values) and monoterpene concentration. Because no curve was available for the unknown monoterpene, this component of the oleoresin was omitted in determining total concentrations of the monoterpenes.

[2] *Use of trade name is for identification and does not constitute endorsement by the Forest Service.*

## Terpenes Detected and Their Concentrations

Seven terpenes were detected in the Douglas-fir wood oleoresin (fig. 2). Six of these were tentatively identified, in the order of their retention times, as $\alpha$-pinene, camphene, $\beta$-pinene, myrcene, 3-carene, and limonene. The remaining compound, number 7, has not been identified. In the 94 trees of this study, $\alpha$-pinene was always the predominant monoterpene. It ranged from 10.4 to 52 percent of the oleoresin content, exclusive of the unknown. Other monoterpenes were present in the following concentrations: $\beta$-pinene, trace to 16.2 percent; myrcene, 0.0 to 4.2 percent; 3-carene, 0.0 to 7.0 percent; and limonene, 0.3 to 8.4 percent. Because camphene was always present in but minute amounts, it was excluded from statistical analyses.

## Monoterpenes and Resistance to Douglas-Fir Beetle

Monoterpenes did not differ qualitatively between trees that survived attack and those that were unattacked (table 1). However, some quantitative differences occurred. At Flathead Lake, concentration of 3-carene differed significantly between trees that resisted attack (groups 3 and 4) and unattacked trees (group 5), while concentration of the unknown monoterpene differed between groups 3 and 5. These two monoterpenes also usually were more highly concentrated in unattacked trees at Salmon River and Moscow Mountain, but the resistance of trees at Moscow Mountain is not known.

The data in table 1 do not directly compare beetle-resistant and susceptible trees. Several problems prevent such a comparison. For example, death after attack was used as proof of susceptibility. Lack of oleoresin exudation pressure, and physiological changes accompanying death made resin sampling impossible or undesirable. Also, the trees that survived may have been attacked by too few beetles to kill them; or the unsuccessful attacks may have disrupted the physiology of the sample trees and affected their monoterpenes. Furthermore, probably not all the unattacked trees in this study were susceptible to beetle attack. The beetle characteristically attacks groups of trees without evident selection. Our inventories of these groups have shown that each contained some survivors. Twenty-two percent of 740 trees attacked in the Flathead Lake area were still alive when examined in 1963, 1 year after attack. Thus, any group of unattacked trees selected at random for sampling is likely to contain some trees that would resist beetle attack. Therefore, our sample of unattacked trees is probably confounded by the presence of resistant trees in the sample.

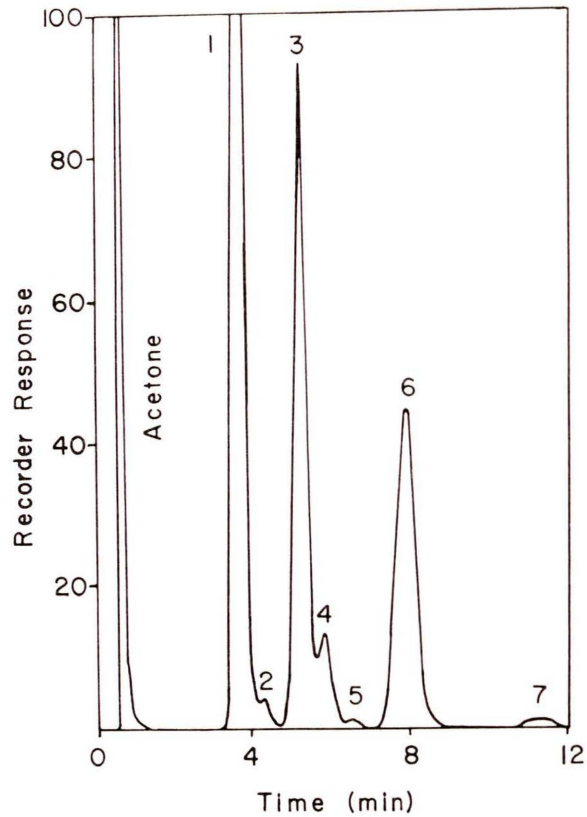


FIGURE 2. — Typical chromatogram of monoterpenes in wood oleoresin of *Pseudotsuga menziesii* var. *glauca* dissolved in acetone (see text).

Table 1.--Mean concentrations of monoterpenes in oleoresin from trees that resisted attack and unattacked trees

| Geographic area and group | Number of trees | Resisted (R) or unattacked (U) | Percent of oleoresin and standard error for- | | | | | | Unknown (integrator units and standard error) |
|---|---|---|---|---|---|---|---|---|---|
| | | | $\alpha$-pinene | $\beta$-pinene | Myrcene | 3-carene | Limonene | Total | |
| Salmon River | | | | | | | | | |
| A 1 | 25 | R | 28.2±1.08 | 2.1±0.3 | 0.8±0.1 | 0.1±0.1 | 2.4±0.2 | 33.6±1.1 | 10.6±1.5 |
| A 2 | 10 | U | 26.7±2.43 | 3.6±1.0 | 0.5±0.1 | 0.6±0.4 | 2.5±0.4 | 33.9±3.2 | 14.0±6.0 |
| Flathead Lake | | | | | | | | | |
| B 3 | 11 | R | 21.7±1.5 | 7.1±1.2 | 0.9±0.1 | 0.1±0.1 | 2.9±0.1 | 32.6±0.8 | 4.5±1.9 |
| B 4 | 15 | R | 25.1±1.0 | 6.9±1.2 | 0.9±0.2 | 0.4±0.2 | 3.2±0.4 | 36.5±1.4 | 11.5±2.6 |
| B 5 | 22 | U | 22.1±1.2 | 4.7±0.6 | 0.4±0.7 | 1.6±0.5 | 2.9±0.3 | 31.6±1.1 | 17.0±3.3 |

Unfortunately, we know no suitable method of testing resistance or susceptibility of Douglas-fir to bark beetle attack. Until such a method is available, biochemical or similar comparisons of trees will be inconclusive.

## Seasonal Variation

Concentration of $\alpha$-pinene and $\beta$-pinene increased significantly between June and October (table 2). In some other species, concentration of monoterpenes has remained constant between seasons (Smith 1964; Mirov 1961). However, Bannister et al. (1959) found that the ratio of $\alpha$-pinene to $\beta$-pinene in resin samples from *Pinus radiata* varied seasonally (seasons not specified) but to a lesser degree than between trees. Analyses of variance between and within seasons showed that the seasonal variation in amounts of $\alpha$-pinene and $\beta$-pinene in Douglas-fir was also significantly less than the between-tree variation. However, the relative concentrations of monoterpenes — especially $\alpha$-pinene — in individual trees did change slightly between seasons. Concentrations of the three other identified monoterpenes were relatively constant between June and October, but the error in measuring low concentrations could mask small seasonal differences. Monoterpenes in *Pinus monticola* (Hanover, in preparation) show a seasonal variation pattern, similar to that of Douglas-fir. Therefore, in comparing monoterpene content of individual trees or in studying monoterpenes in relation to physiological traits, it is desirable to restrict sampling of oleoresin of these species to a uniform time of the year.

## Monoterpenes and Tree Measurements

Simple correlation coefficients of all monoterpenes with 10-year radial growth, diameter, and age were computed for 94 trees by locality. Only combined data for all localities are presented in table 3. Highest positive correlations existed between $\beta$-pinene and limonene, and 3-carene and the unknown. Within localities, strong positive correlations also existed between $\beta$-pinene and limonene and between myrcene and limonene. Monoterpenes were not strongly correlated with tree diameter, age, or growth.

Although only 11 trees were involved at Flathead Lake (Group B-3) it is interesting that all of the identified monoterpenes were highly correlated ($P < 0.01$) either positively or negatively with one another in the group. This result was unique and may reflect a physiological response of trees that repelled bark beetle attack. Possibly, the so-called resistant trees of group 3 were more resistant to attack than the other "resistant" groups. Greater resistance of group B-3 may also be indicated by its low content of 3-carene and unknown terpene as compared with other groups (table 1).

Table 2.--Seasonal variation in the concentrations of five monoterpenes in individual Douglas-fir trees at Flathead Lake

| Tree | Percent of oleoresin for- | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | α-pinene | | β-pinene | | Myrcene | | 3-carene | | Limonene | | Total | |
| | June | Oct. | June | Oct. | June | Oct. | June | Oct. | June | Oct. | June | Oct. |
| 13 | 28.2 | 25.1 | 6.0 | 6.6 | 0.6 | 1.2 | 0.1 | 0.6 | 4.1 | 4.7 | 39.0 | 38.2 |
| 17 | 31.6 | 26.7 | 1.9 | 2.8 | 0.5 | 1.2 | 0.1 | 0.3 | 1.3 | 2.5 | 35.4 | 33.5 |
| 23 | 18.7 | 26.9 | 10.4 | 10.8 | 0.1 | 0.1 | 0.1 | 0.1 | 5.9 | 4.8 | 35.2 | 42.7 |
| 26 | 22.4 | 25.0 | 4.4 | 5.0 | 0.6 | 0.6 | 4.5 | 3.9 | 4.0 | 2.9 | 35.9 | 37.4 |
| 30 | 12.7 | 20.9 | 5.7 | 7.4 | 0.1 | 0.1 | 5.0 | 6.3 | 3.3 | 2.7 | 26.8 | 37.4 |
| 31 | 23.5 | 41.3 | 1.6 | 2.3 | 0.3 | 0.4 | 0.0 | 0.1 | 1.3 | 1.4 | 26.7 | 45.5 |
| 32 | 18.6 | 22.9 | 3.6 | 4.1 | 1.1 | 0.8 | 0.0 | 0.2 | 5.6 | 4.3 | 28.9 | 32.3 |
| 33 | 18.4 | 25.5 | 5.0 | 6.0 | 0.3 | 4.2 | 5.2 | * | 3.5 | 3.2 | 32.4 | 38.9 |
| 34 | 19.1 | 19.6 | 6.8 | 7.7 | 0.1 | 0.1 | 3.7 | 5.8 | 3.9 | 3.7 | 33.6 | 36.9 |
| 42 | 24.2 | 35.7 | 1.3 | 2.4 | 0.1 | 0.1 | 2.5 | 3.9 | 0.9 | 0.5 | 29.0 | 42.6 |
| 51 | 24.4 | 41.3 | 5.2 | 7.6 | 0.6 | 0.1 | 0.1 | 0.6 | 3.6 | 3.0 | 33.9 | 52.6 |
| 63 | 29.3 | 52.0 | 1.9 | 2.3 | 0.1 | 0.1 | 0.0 | 0.1 | 0.6 | 0.8 | 31.9 | 55.3 |
| 64 | 17.8 | 23.0 | 9.0 | 12.1 | 0.5 | 0.1 | 0.1 | 0.5 | 4.7 | 4.8 | 32.1 | 40.5 |
| 69 | 26.0 | 23.3 | 5.5 | 5.5 | 0.9 | 1.7 | 0.1 | 0.4 | 3.3 | 4.3 | 35.8 | 35.2 |
| 94 | 17.6 | 21.0 | 8.2 | 9.2 | 0.1 | 0.1 | 0.1 | 0.3 | 3.5 | 4.3 | 29.5 | 34.9 |
| Average | 22.2 | 28.7 | 5.1 | 6.1 | 0.4 | 0.7 | 1.4 | 1.5 | 3.3 | 3.2 | 32.5 | 40.2 |
| Paired t-test | $P < 0.01$ | | $P < 0.01$ | | NS | | NS | | NS | | $P < 0.01$ | |

* Not determined

Table 3.--Simple correlation coefficients for Douglas-fir monoterpenes and tree characteristics (93 d.f.).

| Monoterpenes | ß-pinene | Myrcene | 3-carene | Limonene | Unknown | 10yr. radial growth | Diameter | Age |
|---|---|---|---|---|---|---|---|---|
| α-pinene | -0.430** | 0.222* | -0.188 | -0.315** | 0.030 | -0.018 | 0.111 | 0.197 |
| ß-pinene | -- | -.011 | -.001 | .657** | .058 | -.005 | -.172 | -.226 |
| Myrcene | | -- | -.386** | .378** | -.024 | -.010 | -.002 | -.049 |
| 3-carene | | | -- | .046 | .557** | .102 | -.242* | -.230* |
| Limonene | | | | -- | .170 | .125 | -.064 | -.245* |
| Unknown | | | | | -- | -.037 | -.101 | -.091 |
| Total | | | | | | .077 | -.034 | -.059 |

* P < .05, r = .202
** P < .01, r = .264

Table 4.--Quantitative variation in Douglas-fir monoterpenes associated with geographic location of trees 1/

| Geographic area | Percent of oleoresin and standard error for- α-pinene | ß-pinene | Myrcene | 3-carene | Limonene | Total monoterpenes | Unknown (integrator units and standard error) |
|---|---|---|---|---|---|---|---|
| A-Salmon R. | 27.8±1.0 | 2.6±0.3 | 0.70±0.08 | 0.27±0.13 | 2.41±0.19 | 33.7±1.2 | 11.6±2.0 |
| B-Flathead L. | [22.9±0.7] | [5.9±0.5] | 0.66±0.07 | [0.86±0.24] 2/ | [3.01±0.21] | 33.4±0.7 | 12.4±1.9 |
| C-Moscow Mtn. | [21.9±1.4] | [5.4±0.8] | 0.55±0.17 | 1.10±0.61 | [4.01±0.59] | 33.0±1.6 | 13.1±4.5 |

1/ Bracketed values differ from unbracketed values at the 0.05 or lower probability levels.

2/ Differs significantly (P < .05) from Salmon River only.

Oleoresin viscosity varied considerably in samples collected for the analyses. However, "very viscous" and "very fluid" resin did not appear to differ in monoterpene composition or content.

## Geographic Differences

If monoterpenes in Douglas-fir are controlled genetically, one could expect an evolution of population differences for these compounds. Such differences might be detectable between populations that are widely separated geographically or by some other barrier to natural crossing. The three populations sampled in this study differed significantly in certain components of their monoterpene composition (table 4). The Salmon River population is distinguished from both Flathead Lake and Moscow Mountain populations by having comparatively higher levels of $\alpha$-pinene but lower levels of $\beta$-pinene, 3-carene and limonene. However, mean total concentration of monoterpene was remarkably constant for all localities.

If these results are representative, they support our hypotheses that the Flathead Lake and Moscow Mountain populations have diverged but little, or that they are more closely related to one another than to the Salmon River population. Further speculation about their evolutionary patterns, based upon monoterpene data, would require more sampling than is reported here. As pointed out by Bannister et al. (1962), interpretation of statistical analyses of population data assumes random sampling and homogeneous variances within the sampled populations. We lack proof of this. Also, we assume a lack of environmental influence on the traits analyzed. Nonetheless, the present study indicates that monoterpenes may be useful in more intensive study of insect resistance, genetics, and physiology of Douglas-fir.

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# GERMINANT SIZE OF JACK PINE IN RELATION TO SEED SIZE AND GEOGRAPHIC ORIGIN

by C. W. Yeatman[1]

The initial size of conifer seedlings is closely related to seed size (Hadders 1963), and seed size is a maternal characteristic that is highly subject to environmental modification (Mergen *et al.* 1964; Righter 1945). The effect of seed weight must be accounted for in critical studies of seedlings which attempt to attribute differences in growth to specific genetic or environmental causes (Duffield 1960; Mitchell 1934, 1939).

This paper reports measurements of seed and germinants (seedlings at the full cotyledon stage) of 87 jack pine (*Pinus banksiana* Lamb.) provenances that included the full range of the species, from the northeastern coast of North America to the Mackenzie river valley in northwestern Canada. Seed weight, seed volume, hypocotyl length, cotyledon length, and cotyledon number were recorded to determine the relative effects of seed weight and seed origin on germinant size.

The seed collections were organized by M. J. Holst, Department of Forestry of Canada, and seed and seedlings have been widely distributed for experimental use in Canada, the United States, and abroad. The results presented here are part of a larger study of the growth of jack pine seedlings in relation to provenance and environment of culture (Yeatman 1964).

## Review of Literature

There are numerous reports in the literature of correlations between seedling size and seed size. The significance of seed size varies with the species, conditions of cultivation and age of seedlings, and range in seed size and in genetic background of the seed samples. By growing seedlings of Scots pine (*Pinus sylvestris* L.) under carefully controlled conditions, Schrock (1964) concluded that seed weight did not greatly influence the length of either hypocotyl or radicle, in contradiction to the earlier conclusion of Schrock and Stern (1953). Mitchell (1934) found a linear relationship between seed weight and the weight of germinants of Scots pine grown in sand without nutrients. He found a similar relationship for white pine (*P. strobus L.*) and applied a coefficient to seedling weight to correct for seed weight. The correction included an adjustment for seed coat weight, which was found to bear a closely linear relation to seed weight (Mitchell 1934, 1939). Hadders (1963) found that hypocotyl length of Scots pine was positively and highly significantly correlated with seed weight ($r = 0.75$), but that the subsequent growth of the epicotyl was not significantly related to seed weight. Reports of significant correlations between seed and seedling size in other coniferous species include: white pine (Spurr 1944), slash pine (*P. elliottii* Engelm.) (Shoulders 1961), ponderosa pine (*P. ponderosa* Dougl. ex Laws.) (Larson 1963), white spruce (*Picea glauca* (Moench.) Voss) (Burger 1964), *Abies* spp. (Mergen *et al.* 1964), Norway spruce (*Picea abies* (L.) Karst.) (Holzer 1960), and Sitka spruce (*P. sitchensis* (Bong.) Carr.) (Burley 1964).

The relationship between seed size and geographic origin has been studied in a number of

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species. Ruden (1963) found a weak correlation between seed size and 11-year plant height of a number of provenances of Norway spruce, but noted that the seed weight differences were largely geographic and that the correlation for single tree progenies from one geographic area was inconclusive. Thulin and Miller (1964) reported that in 26 provenances of European larch (*Larix decidua* Mill.), seed size increased with altitude of seed origin. Wells (1964) found large differences in seed weight between provenances of ponderosa pine. Rudolf (1958) noted that jack pine from the warmer parts of the natural range tend to produce larger seeds than those from colder areas.

Haskell (1961) discussed the possible value of selection by cotyledon number within polycotyledonous tree species. Conifers show a wide range in cotyledon frequency (Butts and Buchholz 1940), which may be of diagnostic value in some instances for the early distinction of species, but of doubtful value within species or for hybrids.

Most attempts to link time of germination, germination percent, and seedling survival with seed size have not been successful (Burger 1964; Shoulders 1961), although Wright and Bull (1962) found that the germination time of European black pine (*Pinus nigra* Arnold) was strongly correlated with seed weight.

Jack pine seeds germinate rapidly when moisture and temperature are adequate (Rudolf 1958). Schantz-Hansen (1941) reported the germination of a number of jack pine provenances within 7 days, with no detectable differences between sources. Some differences in germinative capacity were noted, but these differences bore no relationship to site, age of stand, or geographical location. Critchfield (1957) found significant variation in time of germination between seed sources of the closely related species, lodgepole pine (*Pinus contorta* Dougl. ex Loudon). Seed of northern and high-elevation origin germinated at 20°C more rapidly and more uniformly than that of coastal origin.

## Materials

Representative collections of seed were made from jack pine stands throughout the range of the species in Canada and the United States. The specific place names, latitude, longitude, elevation, and stand descriptions of 100 collections were listed by Holst (1963). The place names and geographic distribution of the 87 provenances used in this study are shown in figure 1.

## Methods

Seed dry-weight, hypocotyl length, length of the largest cotyledon, and cotyledon number were measured for 50 provenances. Dry-weight of seed coat and seed volume were measured on 19 of the 50 seedlots. Seed dry-weight and cotyledon number only were recorded for an additional 37 provenances. Statistical tests were made of the relationships between the provenance mean values of these variables, and with annual normal growing degree-days at the place of origin. The latter index of climate is a temperature-time summation and is principally associated with the growing season measured from the same base temperature, 42°F (5.6°C).

Variations in sampling intensity were unavoidable owing to the small amounts of seed available and to limitations of time, but it is felt that the values presented here are reasonable estimates of the true values for these seed collections.

Seed volume and seed weight were determined from three sub-samples of 50 seed per provenance. Empty seeds were removed before counting by floating in 95-percent ethanol. Cut-tests showed at least 95 percent full seed after separation. The volume of 50 seed was measured with a mercury volume-meter. The seed was dried at 208°F (98°C) for 20 hours before weighing to obtain the dry-weight of 50 seed.

Germinants were grown from fresh seed sown in pots in a greenhouse. The pots contained moist soil covered with a half-inch layer of sand on which the seed was sown. The seed was lightly covered with crushed quartz to conserve moisture. Germination was evident after 7 days and complete when shading was removed at 10 days from sowing.

Seed coats were collected from 19 provenances as they were shed from germinated seed. An attempt was made to avoid the possible bias of collecting only those seed coats shed earlier or later. Three sub-samples provided the basis for calculating the mean dry-weight of 50 seed coats.

The lengths of hypocotyl and longest cotyledon were measured on 16 germinants per provenance (a pair of seedlings was taken at random from each of eight pots). Cotyledon numbers were recorded for 30 seedlings per provenance in another sowing (10 seedlings in each of three pots).

The number of growing degree-days associated with each provenance location was interpolated from the isogram of annual normal growing degree-days above 42°F (5.6°C) published by Boughner and Kendall (1959, Fig. 6) and reproduced here in figure 1. The isolines shown in Michigan and Wisconsin are extensions of the published curves and conform closely to the pattern of "growth degrees" above 50°F (10°C) for the lake States published by the Subcommittee on Forest Tree Seed Collection Zones (1957). The isogram for length of growing season (Can. Dep. Mines and Tech. Surv. 1957), based on a 42°F (5.6°C) temperature limit, is illustrated in figure 1 for comparison. Owing to the wide scatter of meteorological stations, particularly in the northern part of the jack pine range, these generalized curves

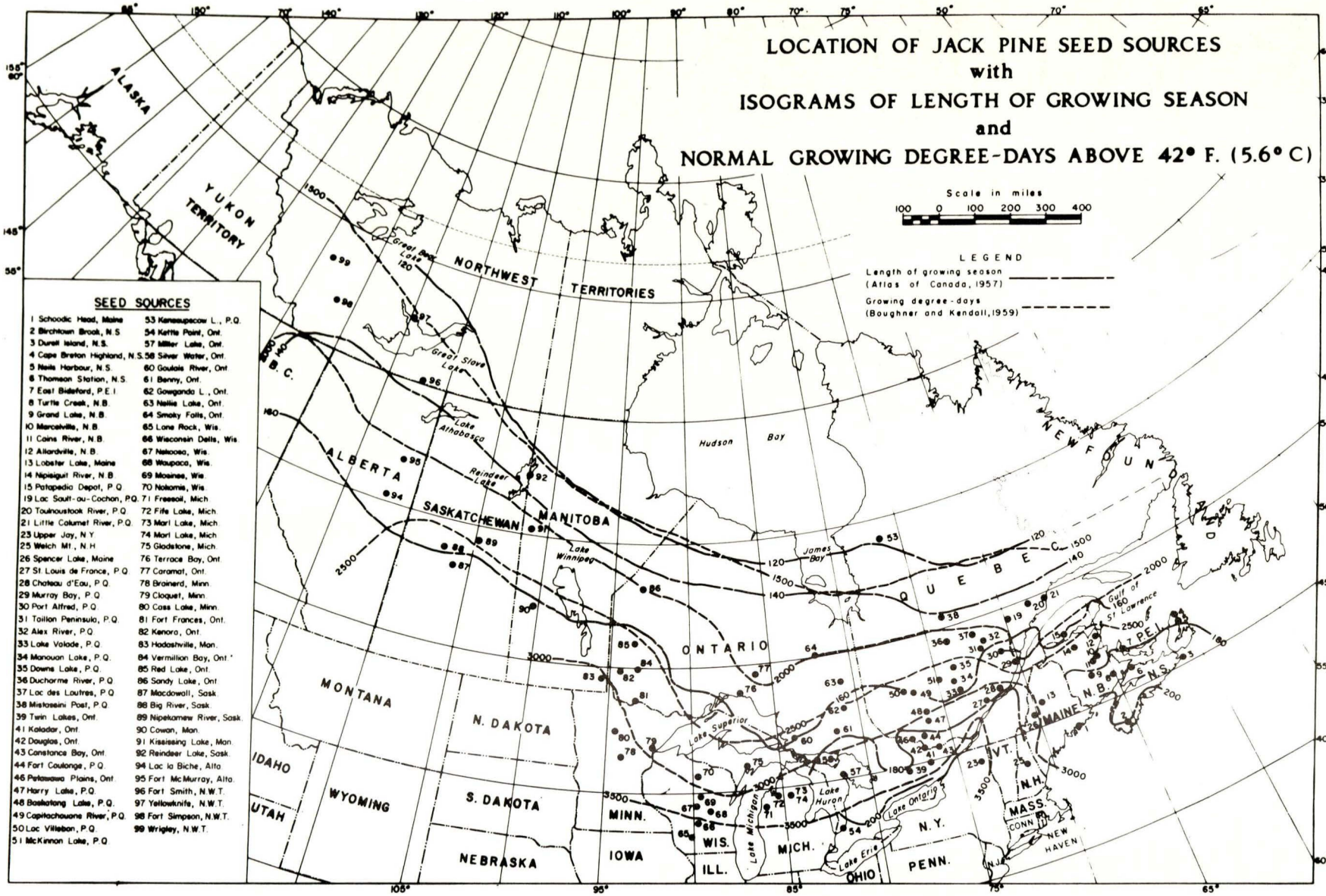


FIGURE 1. — Distribution of jack pine provenances and isograms of climatic indices.

provide a better measure of variation over this wide geographic expanse than do values derived from specific weather stations nearest the places of seed collection.

## Results

The mean values of seed weight and germinant characteristics are listed by provenance number in table 1, together with the values of the climatic index, growing degree-days at the place of seed origin. A value for mean germinant size (germinant index) was derived by adding to hypocotyl length the product of cotyledon number and cotyledon length. The appearance and relative size of the germinants is illustrated in figure 2, representing 30 provenances in six longitudinal groups from East (bottom) to West (top).

It is of interest to record that seed weight (all references herein are to mean dry-weight of 50-seed samples) was highly correlated with seed volume ($r = 0.98^{***}$, $n = 19$). The correlation between seed weight and seed-coat weight was also very highly significant ($r = 0.82^{***}$, $n = 19$). The fact that the two measurements were made on different sub-samples of seed could be expected to add to the error variation in the latter. There was sufficient range in the proportion of seed-coat/whole seed weight, from 31 percent to 42 percent, to suggest that seed-coat thickness does not necessarily change in direct proportion to seed size, but this could only be determined by a more detailed study. The heaviest seed (S. No. 66) in particular had relatively light seed coats.

No differences between provenances were apparent in time or rate of germination. Differences in proportion of filled seed bore no relationship to seed origin or to seed size. In a preliminary trial of seed quality, practically all seed that sank in the liquid separation germinated on moist sand, indicating that filled seed were also viable seed.

The characteristics of the germinants of 50 provenances in relation to seed weight and to growing degree-days are shown in figure 3. The regression between seed weight and growing degree-days (fig. 3A) is significant at the 5-percent level. One provenance (S. No. 41) from an isolated stand in southern Ontario had exceptionally small seed. Calculations of regression including 49 and 86 provenances, and excluding S. No. 41, show that seed weight in general increases with longer and warmer growing seasons. Up to 68 percent of the variation in seed weight is accounted for by the associated growing degree-days as shown below:

| *Number of provenances* | $F_b$ *ratio*[1] | $100r^2$ *(percent)* |
|---|---|---|
| 50 | 6* | 11 |
| [2] 49 | 13*** | 22 |
| 87 | 44*** | 58 |
| [2] 86 | 74*** | 68 |

[1] Levels of significance: *, 5%; ***, 0.1%.
[2] Omitting S.41.

Cotyledon length was highly correlated with cotyledon number ($r = 0.71^{***}$), and the scatter evident in figure 3B was probably exaggerated by the fact that the two measurements were made on different samples of germinants.

Figures 3 C, D, E, and F illustrate the relationships between two dependent variables, cotyledon number and germinant size, and two independent variables, climatic index and seed dry-weight. Dry-weight alone accounts for higher proportions of the variation in the dependent variables than does the climatic index. However, if the exceptional provenance, S. No. 41, is omitted from the calculations, the effect of climatic index alone gains in relative magnitude until it is nearly equal to or better than that of seed weight:

| *Dependent variables* | *Independent variables* Growing degree-days *(percent)* | Seed weight *(percent)* |
|---|---|---|
| Cotyledon number, *a* | 33 | 64 |
| Do. *b* | 49 | 53 |
| Germinant size, *a* | 47 | 54 |
| Do. *b* | 58 | 53 |

*a* = for 50 provenances.
*b* = for 49 provenances, excluding S. No. 41.

The multiple regressions of cotyledon number and germinant size against seed dry-weight and the climatic index taken together are illustrated in figures 4A and 4B. In each case about 75 percent of the variation ($100\ R^2$) is accounted for, and both the independent variables make significant contributions to the functions. The relative magnitudes of the standard regression coefficients ($b'_i$) indicate that seed dry-weight alone contributed twice as much to variation in cotyledon number but that seedling size was affected about equally by each of the independent variables, seed dry-weight and growing degree-days. In both instances, the addition of growing degree-days to the functions contributed significantly more to the variance due to regression than did dry-weight alone ($F = 20.4^{***}$ and $F = 43.8^{***}$ respectively).

## Conclusion

It has been shown that the size of jack pine germinants grown in soil and under normal conditions of light in a greenhouse was dependent upon both seed size and the climate of seed origin, as characterized by growing degree-days. Although seed size was significantly correlated with growing degree-days, the relationship was relatively weak for the 50 provenances that were subjected to detailed analysis. Provenances from areas with

Table 1.--Mean values for seed weight, germinant size, and climatic index for 50 jack pine provenances

| Seed lot number | Dry weight 50 seed [1] | Hypocotyl length | Cotyledons | Length of longest cotyledon | Germinant Index [2] | Growing degree-days at origin | Dry weight 50 seed coats [1] | Volume 50 seeds |
|---|---|---|---|---|---|---|---|---|
| | Mg. | Cm. | No. | Cm. | Cm. | Hundreds | Mg. | $Mm.^3$ |
| 1 | 141 | 3.00 | 3.77 | 2.17 | 11.00 | 26 | - | - |
| 2 | 150 | 3.12 | 4.20 | 2.37 | 13.07 | 29 | - | - |
| 3 | 146 | 3.05 | 3.90 | 2.31 | 12.06 | 28 | 51 | 186 |
| 5 | 166 | 3.15 | 3.90 | 2.42 | 12.59 | 25 | - | - |
| 7 | 162 | 3.39 | 4.30 | 2.58 | 14.48 | 27 | - | - |
| 11 | 158 | 3.46 | 4.13 | 2.61 | 14.24 | 27 | - | - |
| 14 | 162 | 3.14 | 4.10 | 2.41 | 13.02 | 26 | - | - |
| 15 | 172 | 3.34 | 3.90 | 2.59 | 13.44 | 24 | - | - |
| 20 | 168 | 3.29 | 4.07 | 2.48 | 13.38 | 18 | - | - |
| 23 | 196 | 3.51 | 4.30 | 2.96 | 16.24 | 36 | - | - |
| 25 | 179 | 3.49 | 4.27 | 2.51 | 14.21 | 30 | 68 | 231 |
| 26 | 175 | 3.22 | 4.43 | 2.74 | 15.36 | 30 | - | - |
| 27 | 172 | 3.52 | 4.23 | 2.56 | 14.35 | 33 | - | - |
| 29 | 172 | 3.30 | 4.13 | 2.56 | 13.87 | 23 | - | - |
| 32 | 156 | 3.28 | 4.03 | 2.62 | 13.84 | 22 | - | - |
| 33 | 168 | 3.16 | 4.10 | 2.53 | 13.53 | 30 | - | - |
| 35 | 159 | 3.02 | 3.87 | 2.31 | 11.96 | 26 | 60 | 216 |
| 37 | 175 | 3.28 | 4.00 | 2.61 | 13.72 | 21 | - | - |
| 38 | 152 | 2.89 | 3.47 | 2.30 | 10.87 | 19 | - | - |
| 41 | 108 | 3.06 | 3.43 | 2.43 | 11.39 | 35 | 40 | 141 |
| 46 | 178 | 3.69 | 4.57 | 2.83 | 16.62 | 31 | 69 | 222 |
| 47 | 136 | 3.35 | 3.97 | 2.61 | 13.71 | 30 | - | - |
| 50 | 154 | 3.09 | 4.07 | 2.49 | 13.22 | 26 | - | - |
| 53 | 141 | 2.46 | 3.79 | 2.04 | 10.19 | 11 | 56 | 195 |
| 57 | 163 | 3.32 | 4.23 | 2.62 | 14.40 | 32 | - | - |
| 60 | 137 | 3.41 | 3.73 | 2.58 | 13.03 | 26 | - | - |
| 61 | 158 | 3.61 | 3.90 | 2.81 | 14.57 | 27 | - | - |
| 62 | 146 | 3.16 | 3.77 | 2.38 | 12.13 | 25 | - | - |
| 63 | 159 | 3.32 | 3.93 | 2.50 | 13.14 | 23 | 58 | 207 |
| 64 | 153 | 3.09 | 3.93 | 2.31 | 12.17 | 20 | - | - |
| 66 | 255 | 3.54 | 4.47 | 2.94 | 16.68 | 36 | 70 | 294 |
| 70 | 167 | 3.59 | 4.50 | 2.65 | 15.51 | 29 | 64 | 213 |
| 72 | 170 | 3.41 | 4.17 | 2.61 | 14.29 | 32 | - | - |
| 73 | 203 | 3.59 | 4.47 | 2.71 | 15.70 | 33 | 73 | 255 |
| 76 | 141 | 3.05 | 3.67 | 2.26 | 11.34 | 22 | 59 | 186 |
| 77 | 155 | 3.14 | 3.87 | 2.55 | 13.01 | 19 | 59 | 198 |
| 78 | 202 | 3.52 | 4.67 | 2.83 | 16.74 | 33 | - | - |
| 80 | 162 | 3.29 | 4.33 | 2.72 | 15.07 | 32 | - | - |
| 82 | 149 | 3.07 | 4.03 | 2.48 | 13.06 | 28 | 58 | 186 |
| 85 | 131 | 2.96 | 3.87 | 2.30 | 11.86 | 26 | - | - |
| 86 | 152 | 3.14 | 4.00 | 2.48 | 13.06 | 21 | 55 | 192 |
| 87 | 197 | 3.24 | 3.87 | 2.68 | 13.61 | 26 | - | - |
| 89 | 152 | 3.04 | 3.87 | 2.26 | 11.79 | 24 | - | - |
| 91 | 161 | 3.17 | 4.00 | 2.51 | 13.21 | 21 | 62 | 204 |
| 94 | 151 | 2.92 | 3.77 | 2.31 | 11.63 | 23 | - | - |
| 95 | 172 | 2.95 | 4.13 | 2.31 | 12.49 | 21 | 60 | 210 |
| 96 | 180 | 2.98 | 4.00 | 2.44 | 12.74 | 17 | - | - |
| 97 | 131 | 2.38 | 3.43 | 1.99 | 9.21 | 15 | 54 | 168 |
| 98 | 179 | 3.09 | 3.87 | 2.40 | 12.38 | 18 | 71 | 225 |
| 99 | 145 | 2.89 | 3.70 | 2.33 | 11.51 | 17 | 60 | 183 |

[1] Based on 3 subsamples of 50 seed per provenance.

[2] Germinant index is the hypocotyl length in cm. plus the product of cotyledon number and maximum cotyledon length.

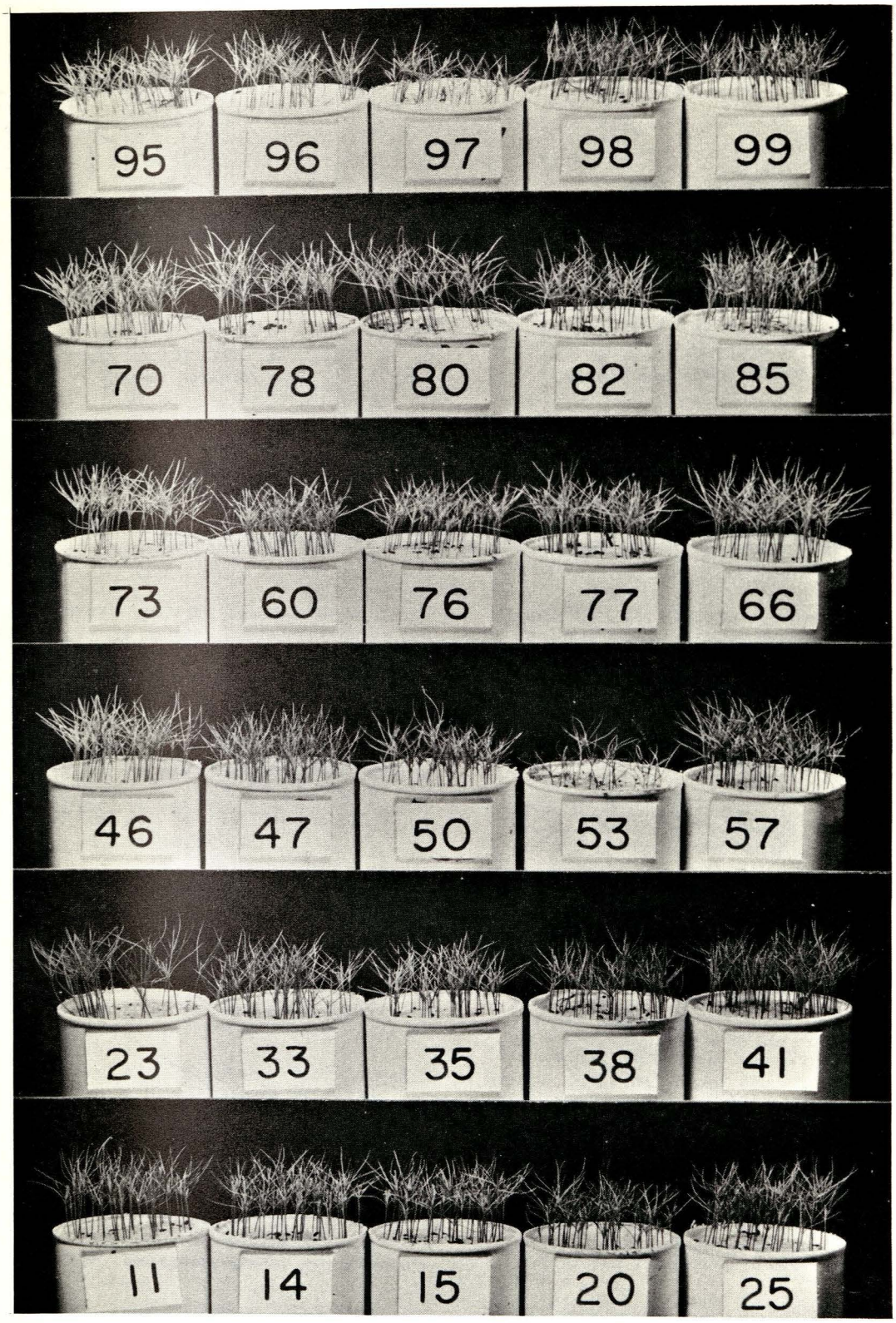


FIGURE 2. — Jack pine germinants of 30 provenances ranging from East (bottom) to West (top).

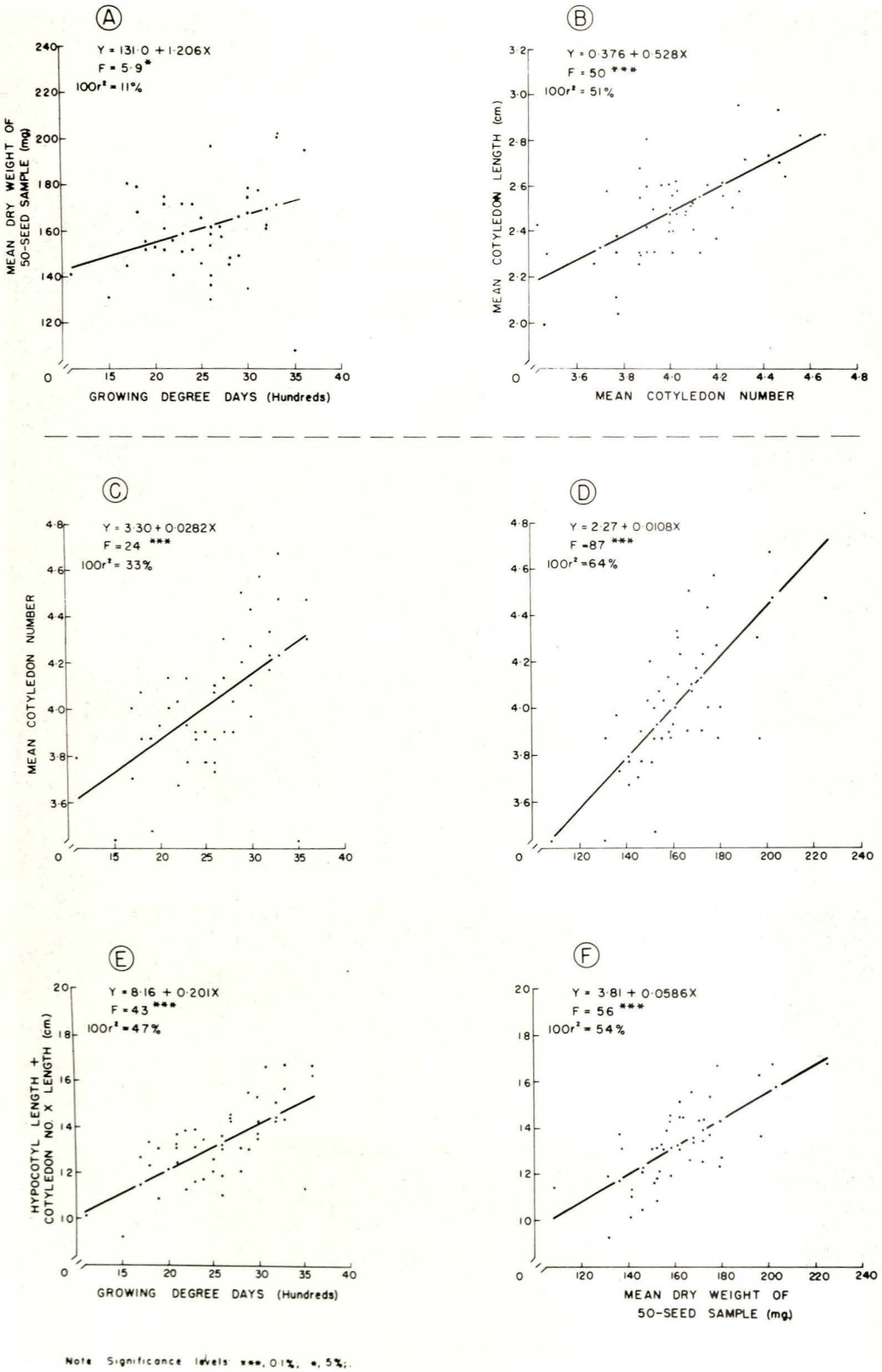


FIGURE 3. — Simple regressions between seed weight, growing degree-days, and characteristics of germinants of 50 jack pine provenances.

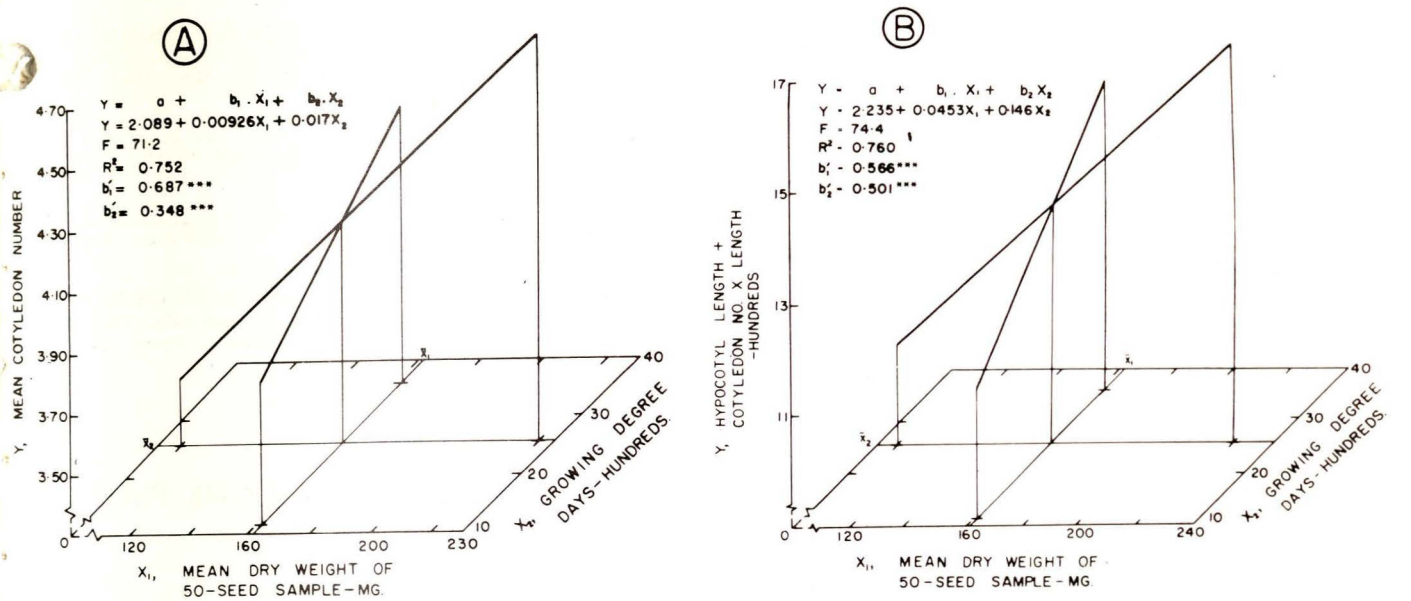


FIGURE 4. — Multiple (2-variable) regressions between seed weight, growing degree-day, and characteristics of germinants of 50 jack pine provenances.

longer and warmer growing seasons were inherently more vigorous in their development of the cotyledonary system, including the hypocotyl, independently of seed weight. Such analyses of germinants grown under very uniform conditions may prove to be useful for early genetic evaluations of vigor and growth potential, especially when compared with the results of experiments dealing with older seedlings.

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# CROSSABILITY AND RELATIONSHIPS OF THE CALIFORNIA BIG-CONE PINES

**by William B. Critchfield**[1]

In the genus *Pinus*, much of the information about species crossability that has accumulated during the past three decades has reinforced ideas about the relationships of pines based on other kinds of evidence. There are some conspicuous exceptions to this generalization, however, and the most thoroughly investigated of these exceptions is Jeffrey pine (*Pinus jeffreyi* Grev. & Balf.), an economically important forest tree of montane California and adjacent parts of Oregon, Nevada, and Baja California. Biochemically, this puzzling species is much like the Sabinianae Loud. (Macrocarpae Shaw), a group of big-cone pines endemic to California: Digger pine (*P. sabiniana* Dougl.), Coulter pine (*P. coulteri* D. Don), and Torrey pine (*P. torreyana* Parry). In its morphology, however, Jeffrey pine is very similar to ponderosa pine (*P. ponderosa* Laws.) and other members of the Ponderosae Loud.,[2] a varied assemblage of yellow pines of western and southwestern North America. In its breeding behavior Jeffrey pine links these two well-defined groups through its ability to cross with members of both groups — *P. ponderosa* and *P. coulteri* — in nature and under controlled conditions.

The contradictory nature of the evidence concerning the relationships of Jeffrey pine led Duffield (1952), in his re-appraisal of the hard pines, to combine the Sabinianae and the Ponderosae into a single group (his group XII). Since 1952 bio-chemical investigations of the pines have been greatly extended, and several new hybrids involving Jeffrey pine or species of the Sabinianae have been produced at the Institute of Forest Genetics at Placerville, Calif. This paper summarizes the results that we have obtained from crossing Jeffrey pine and the Sabinianae with each other and with other pines, and re-evaluates the status of Jeffrey pine and its relatives in terms of this and other recent evidence.

## The Species of the Big-Cone Group

The species included in the Sabinianae form a morphologically and geographically coherent group (Lemmon 1888, Shaw 1914). They are not economically important forest trees, but each species has unique features. Torrey pine has one of the most restricted ranges of any pine, and is the only California hard pine with five needles per fascicle. Digger pine characteristically changes from a single leader to multiple leaders during development, a change that produces the rounded, branchy crown of this species. Coulter pine is noteworthy for its large, heavy, spiny cones, the most massive in the genus. The members of the Sabinianae have in common a coarse branching habit, long coarse needles, several characteristics of the cones and

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[2] *The relationship groups of hard pines outlined by Duffield (1952) have recently been designated as subsections and their names have been brought up to date (Critchfield and Little 1966). The Ponderosae consists of all the species included in Duffield's group XII except the three species making up the Sabinianae (an earlier name of Shaw's Macrocarpae).*

seeds that distinguish the group from the Ponderosae, and paraffin hydrocarbons (heptane, nonane, undecane) in their wood oleoresin, a feature which they share with Jeffrey pine. Another biochemical characteristic that distinguishes the Sabinianae and Jeffrey pine from several members of the Ponderosae is the presence of alkaloids in the needles (Tallent *et al.* 1955).

With the exception of Torrey pine, which is geographically isolated from all related species, there is a high degree of sympatry among the Sabinianae, Jeffrey pine, and ponderosa pine (the principal California representative of the Ponderosae). In the upper part of its elevational range, Digger pine is commonly mixed with ponderosa pine. At a few places it meets the higher-elevation Jeffrey pine, usually in association with ponderosa pine as well[3]. Ponderosa pine is mixed with Jeffrey pine in many parts of California, and in the mountains of southern California both species occur with Coulter pine separately or together[4]. Coulter pine occurs with Digger and ponderosa pine in parts of the central Coast Ranges, and with Jeffrey and Digger pines in one locality (San Benito County).[3] The distribution of these pines is shown by Critchfield and Little (1966).

Coulter pine has been exploited in the genetic improvement of Jeffrey pine. Young Jeffrey pines are sometimes badly damaged by the pine reproduction weevil (*Cylindrocopturus eatoni* Buch.), especially in plantations. Coulter pine has proven to be completely resistant to this insect under forced attacks (Miller 1950). Backcrosses of natural Jeffrey-Coulter hybrids to Jeffrey pine have also exhibited a high level of resistance under forced attacks (Miller 1950; Smith 1960) and in field tests (Hall 1959). This resistance of the backcross hybrids led Region 5 of the U.S. Forest Service to start producing them on a large scale several years ago (Libby 1958).

## Previous Reports of Natural and Artificial Hybrids

Although Jeffrey pine has generally been treated as a separate species in recent years, opinion concerning its status was divided in the past. Lemmon (1888, 1890) and Sudworth (1908) considered it a distinct species, but other botanists regarded it as a variety of ponderosa pine. Shaw (1914) noted: "Most observers discover many intermediate forms between this variety and the species," and Jepson (1910) stated, ". . . the transition forms in the intermediate region are quite as numerous and occupy as extensive an area as the Jeffrey Pine itself."

The findings of Schorger and others (see Mirov 1961) that the turpentine of Jeffrey pine is completely different in composition from that of ponderosa pine may have helped to define Jeffrey pine as a separate species. The first definite report of a hybrid between the two species was in 1929. A field survey in northeastern California turned up a single tree, intermediate in morphology, with turpentine consisting of both heptane, like Jeffrey pine, and monoterpenes, like ponderosa pine (Mirov 1929). At about the same time, the two species were crossed reciprocally at the Institute of Forest Genetics, then called the Eddy Tree Breeding Station (table 1).

The extent of hybridization between ponderosa and Jeffrey pines in several mixed populations was recently investigated by Haller (1962). Sampling more or less at random but including some variant trees, he found only a single putative $F_1$ and a few possible hybrid derivatives, a small fraction of the sampled trees. He concluded that gene exchange between these two species is very limited.

Jeffrey and Coulter pines, because of their pronounced morphological differences, were not considered to be at all closely related until 1937, when a natural hybrid of these species was found in the mountains of southern California (Libby 1958). In 1939 this tree was backcrossed to Jeffrey pine, and a few years later the first artificial $F_1$ hybrids between Jeffrey and Coulter pines were produced (table 1).

Natural hybridization between Jeffrey and Coulter pines was investigated by Zobel (1951),[5] who made an extensive search for hybrids in several mixed populations. He found five putative $F_1$ hybrids, a few possible backcrosses to Jeffrey pine, and fairly numerous possible backcrosses in the other direction, grading more or less continuously into Coulter pine. Like Haller, Zobel concluded that natural hybridization has been ineffective in altering the characteristics of either species. A few years later, Libby located additional natural Jeffrey x Coulter hybrids (Libby 1958).

Verified hybrids have also been reported between Jeffrey pine and two other members of the Ponderosae: Washoe pine (*Pinus washoensis* Mason & Stockwell) and Montezuma pine (*P. montezumae* Lamb.) (table 1). An early report of a

[3] *Griffin, James R. Intraspecific variation in Pinus sabiniana Dougl. Ph.D. thesis, Univ. Calif., Berkeley. 274 pp., illus. 1962.*

[4] *Zobel, Bruce J. The natural hybrid between Coulter and Jeffrey pines. Ph.D. thesis, Univ. Calif., Berkeley. 114 pp., illus. 1951.*

[5] *See also, Zobel, Bruce J. The natural hybrid between Coulter and Jeffrey pines. Ph.D. thesis. Univ. Calif., Berkeley. 114 pp., illus. 1951.*

Table 1.--Hybrids of Sabinianae pines and Jeffrey pine produced at the Institute of Forest Genetics, Placerville

| Parents (Pinus-) | | Pollination year first produced | Year planted | First cited |
|---|---|---|---|---|
| Female | Male | | | |
| sabiniana | coulteri | 1958 | 1960 | This paper |
| sabiniana | torreyana | 1958 | 1960 | This paper 1/ |
| jeffreyi | coulteri | 1944 | 1946 | Righter and Duffield 1951 |
| jeffreyi | ponderosa | 1929 | 1933 | Schreiner 1937 |
| ponderosa | jeffreyi | 1929 | 1933 | Weidman 1947 |
| jeffreyi | montezumae | 1951 | 1953 | Liddicoet and Righter 1960 |
| jeffreyi | engelmannii | 1955 | 1962 | This paper |
| jeffreyi | washoensis | 1948 | 1950 | Liddicoet and Righter 1960 2/ |

1/ An earlier cross (1947), planted in 1949, was removed in 1953. These putative hybrids are mentioned in the unpublished Annual Report for 1949, California Forest and Range Exp. Sta., 1950, on file at Pacific Southwest Forest and Range Exp. Sta., U.S. Forest Serv., Berkeley, Calif.

2/ Also listed as verified hybrid in unpublished Annual Report for 1950, California Forest and Range Exp. Sta., 1951, on file at Pacific Southwest Forest and Range Exp. Sta., U.S. Forest Serv., Berkeley, Calif.

successful cross between Jeffrey and Montezuma pines (Duffield 1952)[6] appears to be in error; the earliest verifiable hybrids between these two species were produced by 1951 pollinations (table 1).

Two non-existent hybrid combinations involving members of this group are sometimes cited. One of these false reports appears to have had its origin in a statement by Austin (1927) that sound seed had been obtained from a 1926 cross between ponderosa and Digger pines. This seed, probably the result of pollen contamination during this first season of controlled breeding at Placerville, has been reported as a ponderosa x Digger pine hybrid by Richens (1945) and others. Richens also reported a hybrid between Coulter and ponderosa pines, but the source of this erroneous information is unknown.

## Parent Trees

Several of the species concerned in the crosses summarized here are native to the vicinity of Placerville, which is in Eldorado County in the central Sierra Nevada. About 20 Digger pines, widely scattered through the foothill zone of western Eldorado County, were used as parents. Nearly all of the Jeffrey pine crosses were made with about 55 trees growing at higher elevations in and near eastern Eldorado County, and most of the 60 or more ponderosa parents were in native stands in central Eldorado County. Arizona pine (*P. ponderosa* var. *arizonica* (Engelm.) Shaw), which is treated separately here, was represented by only three arboretum trees, all from the same collection in the Chiracahua Mountains of southern Arizona. The Washoe pine crosses involved six parent trees growing in two localities on Mount Rose, Nev.

The other species considered here were principally represented by trees growing in the arboretum. About a dozen Coulter pines were used, originating in several localities from San Diego to Contra Costa Counties. Torrey pine does not flourish at Placerville, and only about five trees were used as parents. The seven parent trees of Apache pine (*P. engelmannii* Carr.) originated from two seed collections in the Chiracahua Mountains. Durango pine (*P. durangensis* Martinez) was represented by a single tree from the state of Durango, Mexico. Ten trees of Montezuma pine were used, most of them growing in the arboretum and originating from several different collections of Mexican or unknown origin. Three trees growing in natural stands in central Mexico were used as pollen parents.

Most of the crosses were made on Jeffrey and ponderosa pines, and crosses between these two species were usually made on the later-flowering Jeffrey pine. Digger pine was not used more often as a female parent because it flowers earlier than

[6] *See also U. S. Forest Service, Calif. Forest and Range Exp. Sta. Unpublished Report for 1950. On file at Pacific Southwest Forest and Range Exp. Sta., U. S. Forest Serv., Berkeley, Calif.*

any of the other species considered here except Torrey pine. Coulter pine produces abundant pollen from an early age at Placerville, but its cone production is rather limited there. Torrey pine and the southwestern and Mexican pines are unpredictable and generally sparse in their flowering in Placerville.

## Methods

The results summarized here cover a 35-year period: the pollinating seasons of 1929 through 1963. The techniques used to control pollination in pines have been described by Cumming and Righter (1948). Numbers of sound and hollow seed were often determined by weighing the seed in the 1930's, but since then a Clipper Mill has been used for separating sound and hollow seed.

Many crosses involving these species have been omitted from this summary, including all crosses that failed to yield cones and all crosses for which data are not complete (numbers of strobili pollinated, cones harvested, and sound and hollow seed). Crosses that produced seed which was badly damaged by insects (more than 10 percent) have been omitted. Also omitted are nearly all of the crosses involving pollen that had been refrigerated for a year or more. Since 1961 we have deep-frozen all of our pollen at Placerville, and a few crosses utilizing year-old frozen pollen are included here. Also included are the crosses between Digger and Coulter pines, all of them made with year-old refrigerated pollen, since one of them yielded the single Digger x Coulter hybrid so far obtained.

With these exceptions, the data summarized here include all interspecific crosses involving the three species of Sabinianae, and all crosses of Jeffrey pine with members of the Sabinianae and Ponderosae. Also summarized are all intraspecific crosses of Coulter, Washoe, and Apache pines, and partial but representative data on intraspecific crosses of Jeffrey, ponderosa, and Digger pines. A comprehensive evaluation of interspecific crosses between members of the Ponderosae is outside the scope of this paper, but rough estimates of their crossability are presented as a basis for evaluating the status of Jeffrey pine in relation to the Ponderosae.

Several species combinations have produced sound seed but not verified hybrids. Single crosses of Washoe x Jeffrey and Jeffrey x Arizona pines have each yielded a single seed which failed to germinate. Three other species combinations (Jeffrey x Torrey, ponderosa x Coulter, and Washoe x Coulter) have yielded from one to a few sound seeds, but our records do not indicate the fate of these small seed lots.

An *attempt*, as used here, refers to the pollination of a single female parent with pollen from a single male parent or with a mixture of pollen from several male parents. The minimum number of tree x tree combinations attempted has been estimated by assuming that two male parents have contributed to pollen mixes of unspecified constitution. *Crossability*, as quantitatively expressed here, is the mean sound seed yield from an interspecific combination expressed as a percent of the mean sound seed yield of crosses within the maternal-parent species. In those few instances where two species have been crossed reciprocally (e.g. Jeffrey and Washoe pines), the two crossabilities have been averaged after weighting by the number of attempts.

## Crossing the Sabinianae Pines

Despite the morphological similarity of Coulter and Digger pines, this species combination appears to be decidedly less crossable than Digger and Torrey pines. Crosses of Coulter x Digger have not yielded any hybrids (table 2), and the reciprocal combination has yielded a single tree.

Coulter and Digger pines differ chiefly in cone and seed characteristics and wood-resin composition. The hybrid is too young to produce cones, but resin composition has proven to be conclusive in establishing its identity. The low-boiling-point fraction of Digger pine resin consists almost entirely of heptane, plus a small amount of nonane (Mirov 1961; Williams and Bannister 1962). The same fraction of Coulter pine resin is made up principally of $\alpha$-pinene, $\beta$-phellandrene, and myrcene, with lesser amounts of several other compounds including the three paraffin hydrocarbons known to occur in *Pinus*: heptane, nonane, and undecane (Mirov 1961; Williams and Bannister 1962; Smith[7]). The resin of the hybrid tree, analyzed by gas-chromatographic techniques, is clearly intermediate. It contains large amounts of heptane and $\alpha$-pinene, a lesser amount of $\beta$-phellandrene, and small amounts of several other constituents of Coulter pine resin (table 3). In other respects the tree does not differ greatly from young Digger pines.

In the few attempts that we have made, Digger pine combines much more readily with Torrey than with Coulter pine, although it differs from Torrey pine in cone form, number of needles per fascicle (usually three, compared to five in Torrey), and other characteristics. Three of the five attempts to cross these two species (table 2) have yielded 3 to 8 sound seed per cone. A large number of putative hybrids was obtained from the first of these, a cross made in 1947. These trees were judged to be nonhybrids at 5 years of age and were removed. The other two attempts were made in 1958, using other parent trees. Both crosses were made with pollen from a single Torrey pine.

[7] *Smith, Richard H. Variations in the wood resin monoterpene composition of Pinus jeffreyi, P. washoensis, P. coulteri, P. contorta. In manuscript at Pacific Southwest Forest and Range Exp. Sta., Berkeley, Calif.*

Table 2.--Summary of Institute of Forest Genetics crosses involving Sabinianae pines and P. jeffreyi, 1929-1963

| Male parent | Female parent | | | | | |
|---|---|---|---|---|---|---|
| | sabiniana | coulteri | jeffreyi | ponderosa | washoensis | engelmannii |
| torreyana | 5(6)/4<br>42, 69<br>2.1/60.4 | 4(7)/0<br>31, 29<br>0/122.3 | 3(6)/1<br>29, 48<br>0.4/71.9 | 3(3)/0<br>13, 77<br>0/42.1 | | |
| sabiniana | 1/ 16(16)/16<br>80, 61<br>87.4/99.1 | 8(15)/0<br>25, 44<br>0/98.7 | 4(4)/0<br>23, 57<br>0/71.2 | 6(12)/0<br>42, 55<br>0/55.2 | | 1(1)/0<br>10, 100<br>0/13.0 |
| coulteri | 2/ 8(12)/2<br>61, 41<br>0.1/45.0 | 1(3)/1<br>10, 70<br>86.1/106.9 | 29(41)/17<br>1112, 61<br>7.2/105.9 | 13(17)/2<br>290, 39<br><0.1/48.2 | 4(11)/1<br>61, 36<br><0.1/69.1 | |
| jeffreyi | | | 1/ 20(25)/20<br>164, 79<br>142.3/185.3 | 8(12)/2<br>83, 46<br><0.1/49.4 | 3(9)/1<br>23, 57<br><0.1/65.5 | |
| ponderosa | | | 32(36)/27<br>854, 40<br>4.1/134.7 | 1/ 29(43)/29<br>278, 45<br>52.1/57.8 | | |
| ponderosa var. arizonica | | | 4(5)/1<br>127, 26<br><0.1/141.0 | | | |
| washoensis | | | 1(2)/1<br>14, 71<br>3.4/172.8 | | 3(7)/3<br>27, 52<br>65.6/110.6 | |
| engelmannii | | | 17(19)/6<br>367, 73<br><0.1/158.6 | | | 2(3)/2<br>13, 85<br>46.8/54.3 |
| durangensis | | | 1(1)/0<br>4, 75<br>0/75.3 | | | |
| montezumae | | 1(1)/0<br>4, 25<br>0/127 | 6(13)/3<br>199, 63<br><0.1/116.5 | | | |

Legend:

Number of attempts — 0 (0)/ 0 — Minimum number of tree x tree combinations
Number of female strobili pollinated — 0, 0 — Number of attempts producing sound seed
Mean number of sound seed per cone — 0.0/0.0 — Percent of strobili producing cones
Mean total number of seed per cone

Successful interspecific cross (verified hybrids obtained)

Footnotes:
1/ Not all crosses included.
2/ Year-old pollen.

They yielded trees that have been identified as hybrids on the basis of their oleoresin composition. The low-boiling-point fraction of Torrey pine oleoresin consists mostly of limonene, with small amounts of heptane, nonane, undecane and other compounds (Mirov 1961; Williams and Bannister 1962). The resin of all five putative hybrids available for sampling contains large amounts of both limonene, like Torrey pine, and heptane, like Digger pine, plus small amounts of nonane, undecane, and other compounds (table 3).

In other respects the Digger-Torrey hybrids are nearly indistinguishable from young Digger pines. In number of needles per fascicle, a characteristic that might be expected to differentiate the hybrids from both parent species, Digger pine is partly dominant. In a 10-fascicle sample of each of the 5 hybrids listed in table 3, 2 had exclusively three-needled fascicles and the other 3 had mixtures of three- and four-needled fascicles. None had any five-needled fascicles. Thus all of the hybrids are within the range of Digger pine itself, which often has more than three needles per fascicle.[8] This

[8] *Griffin, James R. Intraspecific variation in Pinus sabiniana Dougl. Ph.D. thesis, Univ. Calif., Berkeley, 274 pp., illus. 1962.*

Table 3.--Low-boiling-point constituents of wood oleoresin of Pinus sabiniana x coulteri, P. sabiniana x torreyana, and their parents 1/

| Tree No. | Parentage of hybrids | Constituent (percent) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | hep-tane | nonane | α-pinene | un-decane | cam-phene | β-pinene | 3-carene | sabin-ene | myr-cene | limon-ene | β-phell-andrene |
| | | | | | | P. coulteri | | | | | | |
| C1-V30 2/ | -- | 2.4 | 1.5 | 40.3 | 4.3 | 0.4 | 2.1 | tr | 1.5 | 21.0 | 3.9 | 22.5 |
| | | | | | | P. sabiniana x coulteri | | | | | | |
| SC1-1 | S-E1d-13-1 x C1 (V28 + V30) | 34.8 | 1.8 | 43.0 | 4.5 | 0.3 | 1.8 | 0.8 | tr | 0.3 | 0.3 | 12.4 |
| | | | | | | P. sabiniana | | | | | | |
| S-E1d-13-1 | -- | 93.3 | 6.7 | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| S-E1d-13-2 | -- | 96.0 | 4.0 | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| | | | | | | P. sabiniana x torreyana | | | | | | |
| STo-17 | S-E1d-13-2 x To-V27 | 43.9 | 5.9 | 2.8 | 7.8 | -- | 0.3 | 0.6 | -- | 2.1 | 36.6 | -- |
| STo-21 | Do. | 45.4 | 1.4 | 4.2 | 4.3 | -- | tr | tr | -- | 1.4 | 43.3 | -- |
| STo-27 | S-E1d-13-1 x To-V27 | 56.0 | 1.2 | 1.9 | 4.7 | -- | -- | 1.2 | -- | 1.5 | 33.5 | -- |
| STo-29 | Do. | 23.1 | 1.4 | 11.2 | 7.0 | -- | -- | -- | -- | 1.4 | 55.9 | -- |
| STo-32 | Do. | 26.6 | 1.6 | 2.4 | 5.7 | -- | -- | -- | -- | 1.6 | 62.1 | -- |
| | | | | | | P. torreyana | | | | | | |
| To-V27 | -- | 3.2 | 2.4 | 1.8 | 7.7 | -- | tr | -- | -- | 2.7 | 82.2 | -- |

1/ Percentages are based on constituents listed. Pentane and acetone solutions of resin were analyzed on an 8-ft. 10-percent β, β' oxydipropionitrile column under the following conditions: injector temperature 140°C, column temperature 55°C, thermal detector temperature 165-170°C, filament current 170 ma, and helium volume 63 to 71 ml per minute.

2/ Data supplied by R. H. Smith. The other P. coulteri used in pollen mix (C1-V28) was removed in 1960.

may have been true also of the putative hybrids from the 1947 cross, which were evaluated when resin analysis of young trees was not yet possible, and for this reason the 1947 cross has been included in the summarized data.

With the notable exception of Jeffrey and Coulter pines, all attempts to cross the Sabinianae with other pines have failed (table 2). Most species combinations have been tried on a rather small scale, but collectively they show that there is a strong degree of isolation between the Sabinianae and the Ponderosae, so far bridged only by Jeffrey pine. One combination attempted on a fairly large scale, ponderosa x Coulter pine (13 attempts yielding 290 cones), has produced a few sound seed on two occasions. Although the fate of these seeds is unknown, further trials of this combination may be warranted.

## Crossing Jeffrey Pine with the Sabinianae an Ponderosae Pines

All crosses between Jeffrey pine and Digger and Torrey pines have been unsuccessful. These crosses, which have not been tried on a large scale, also show a marked reduction in the yield of cones and total seed compared to intraspecific crosses (table 2). These measures of incompatibility are difficult to evaluate in the absence of control crosses, however.

In contrast, crosses between Jeffrey and Coulter pines have averaged 7.2 sound seed per cone. This is a higher yield of sound seed than any other species combination involving Jeffrey has produced (table 2). It is heavily biased, however, by repeated crosses made on an otherwise unremarkable Jeffrey pine. This tree, unlike its neighbors in the stand, has consistently yielded 20 to 50 sound seed per cone irrespective of the Coulter parent. It has produced more than four-fifths of the sound seed obtained in the Jeffrey x Coulter crosses summarized here. A less biased estimate of the average sound seed per cone for Jeffrey x Coulter — 3.4 — includes only the first cross made on this tree.

With the exception of the Jeffrey x Coulter combination, crosses between Jeffrey pine and species of the Ponderosae have been on a much larger scale than other attempts to cross this species (table 2). Although four different hybrid combinations have been obtained in all (table 1), the genetically controlled reproductive isolation of Jeffrey pine from Southwestern U.S. and Mexican representatives of the Ponderosae appears to be almost complete. Only three Jeffrey x Montezuma hybrids have been produced, and only a single Jeffrey x Apache hybrid.

The ability of Jeffrey pine to cross with the sympatric ponderosa pine is considerably greater. The relatively few attempts in which ponderosa pine has been used as a female parent have not been very successful, but much larger scale attempts in the opposite direction have yielded an average of 4.1 sound seed per cone (table 2). This

compares favorably with the corrected average of 3.4 sound seed for Jeffrey x Coulter combinations.

How do these two species combinations — Jeffrey x Coulter and Jeffrey x ponderosa — compare in other measures of compatibility? A larger proportion of attempts to cross Jeffrey x ponderosa has yielded sound seed: 84 percent compared to 59 percent for Jeffrey x Coulter. And the Jeffrey x ponderosa crosses have also yielded appreciably higher total numbers of seed per cone: 135 compared to 106 for Jeffrey x Coulter and 185 for within-Jeffrey crosses. But the level of conelet abortion is lower in Jeffrey x Coulter combinations. They produced cones from 61 percent of the strobili pollinated, compared to only 40 percent for Jeffrey x ponderosa crosses and 79 percent for within-Jeffrey crosses. These data are not critical, but in conjunction with the sound seed yield they indicate that the compatibility of Jeffrey pine with Coulter and ponderosa pines is of the same order of magnitude.

The interspecific hybrids of Jeffrey pine are highly fertile, like most other pine hybrids but unlike interspecific hybrids in many other groups of plants. Meiotic irregularities during microsporogenesis were about equally uncommon in trees of the parent species and in representative Jeffrey-Coulter and Jeffrey-ponderosa $F_1$ hybrids in Saylor's analysis.[9] The production of aborted pollen grains by these Jeffrey pine hybrids has not been critically investigated, but routine observations indicate that they are generally few. This was not the case in the first natural Jeffrey x Coulter hybrid used in breeding; about half of its pollen aborted (Libby 1958). Many of its wind-pollinated and backcross offspring growing at Placerville also show high levels of pollen abortion.

The ability of these Jeffrey pine hybrids to yield sound seed in backcrosses is also remarkably high, considering the comparatively strong reproductive barriers that separate Jeffrey pine from other species. A Jeffrey x ponderosa hybrid used as female parent in backcrosses to unrelated individuals of ponderosa and Jeffrey yielded only about 10 sound seeds per cone with ponderosa but a remarkable 95 sound seeds per cone with Jeffrey. The Jeffrey x Coulter $F_1$ hybrids began producing pollen only a few years ago, and the backcrosses that have so far been made to Jeffrey pine have suffered heavy seed losses from insects. But one set of crosses involving three Jeffrey female parents and a mixture of pollen from three hybrids produced an average of 133 sound seed per cone, 93 percent of the control-cross seed yield. Other backcrosses, heavily damaged by seed insects and not involving pollen mixtures, have yielded much less sound seed — from 0 to 66 per cone.

[9] *Saylor, Leroy C. Chromosome behavior and morphology in species and interspecific hybrids of Pinus. Ph.D. thesis, N. C. State Coll., 128 pp., illus. 1962.*

## Discussion

Although most of the California pines considered here are associated in natural stands, only two hybrid combinations have been encountered in nature: hybrids of Jeffrey with Coulter and ponderosa pines. Ponderosa, Digger, and Coulter pines are all partly sympatric, but they are apparently prevented from hybridizing by strong reproductive barriers reinforced by differences in flowering time. Near Placerville, native Digger pine flowers more than 3 weeks before native ponderosa pine and about a month before planted Coulter pine. Phenological differences of this magnitude probably do not eliminate the possibility of cross-pollination between species, but they must reduce it to the level of an uncommon event.

The limited crossability of Jeffrey pine with Coulter and ponderosa pines helps to explain the rarity of natural hybrids between these species. In both cases the reproductive barriers that restrict crossing are accompanied by differences in flowering time. At Placerville native ponderosa pine flowers a little more than 2 weeks before planted Jeffrey pine, and Haller (1962) noted a difference of 2 weeks to a month in pollen-shedding time in natural stands. Planted Coulter pine flowers only 9 days before planted Jeffrey pine at Placerville, but a few observations suggest that the difference may be somewhat greater in nature. It is impossible to compare the frequency of these two natural hybrids of Jeffrey pine because of the differing approaches to sampling taken by Zobel (1951) and Haller (1962). Zobel's sampling was oriented around natural hybrids that had been located after an extensive search, whereas Haller sampled mixed stands nearly at random. However, Zobel's observation that he found no indications of natural hybridization in many mixed stands, compared to Haller's finding that all three of his sample areas showed indications of natural hybridization, suggests that hybridization between Jeffrey and ponderosa pines may be more common in nature.

The anomalous characteristics and crossing behavior of Jeffrey pine have produced a considerable diversity of recent opinion concerning its relationships. Mirov (1961), impressed by the chemical similarity of its turpentine to that of the Macrocarpae (i.e. Sabinianae) pines, transferred it from the Australes of Shaw (which includes the Ponderosae) to the Macrocarpae. He noted, however, that he considered it to occupy an intermediate position between the Macrocarpae and the western Australes (i.e. Ponderosae). An opposing view was expressed by Zobel in his study of Jeffrey and Coulter pines and their hybrids (1951). He left Jeffrey pine in the Australes and re-emphasized the distinctness of the Macrocarpae and Australes groups. Still a third viewpoint was adopted by Duffield (1952) at a time when the only hard pines

known to have paraffin hydrocarbons in their resin were the Macrocarpae and Jeffrey pine. He considered the morphological similarities of Jeffrey and ponderosa pines too great to warrant transferring Jeffrey pine to the Macrocarpae, but on biochemical and crossability grounds he eliminated the Macrocarpae and provisionally grouped these species with the western Australes. At the same time he observed, ". . . . the group Macrocarpae is coherent biochemically, geographically, and morphologically."

Since 1952 the biochemical argument for associating Jeffrey pine with the Sabinianae pines has lost some of its force. Paraffin hydrocarbons have been found to occur sporadically in several members of the Ponderosae. Mirov (1961) reported that ponderosa pine growing in Santa Cruz County, Calif., has a small amount of undecane in its turpentine; Montezuma pine growing in the state of Chiapas, Mexico, has a substantial amount of heptane (8 percent); and *P. oaxacana* Mirov, a weakly segregated relative of the Mexican *P. pseudostrobus* Lindl., has both heptane (21 percent) and undecane (1.3 percent) in its turpentine. It should also be re-emphasized that neither Coulter nor Torrey pine turpentine contains more than 10 to 15 percent of the paraffin hydrocarbons; their turpentine consists predominantly of monoterpenes.

Arguments for associating Jeffrey pine with the Sabinianae on the grounds of crossability have also lost some of their persuasiveness in the past decade. The coherence of the Sabinianae has been strengthened by successful crosses in two of the three possible combinations. At the same time, Jeffrey pine has been more firmly linked to the Ponderosae by successful crosses with four members of the group. Finally, additional attempts to cross species of the Sabinianae and Ponderosae groups have been unsuccessful, and the two groups are still bridged only by the ability of Jeffrey and Coulter pines to cross (fig. 1).

The strongest arguments for retaining the Sabinianae as a separate group, and for excluding Jeffrey pine from it, are the numerous morphological features that distinguish Coulter, Digger, and Torrey pines from Jeffrey pine and the species of the Ponderosae. Some of these were mentioned by Shaw (1914): the long stout leaves, the large cones, and the basally thickened seed wing. Unifying features noted by Lemmon (1888) are the long stout conelet peduncles and the very thick, hard seed coats. Other definitive characteristics of the group are the delayed and gradual cone opening, the persistence of the cones on the tree, the large seeds, and the numerous cotyledons (Sudworth 1908; Jepson 1910). The density of Torrey pine cones has not been investigated, but both Coulter and Digger pines have very dense cones; cone specific gravity averages 0.70 to 0.85 in Digger pine

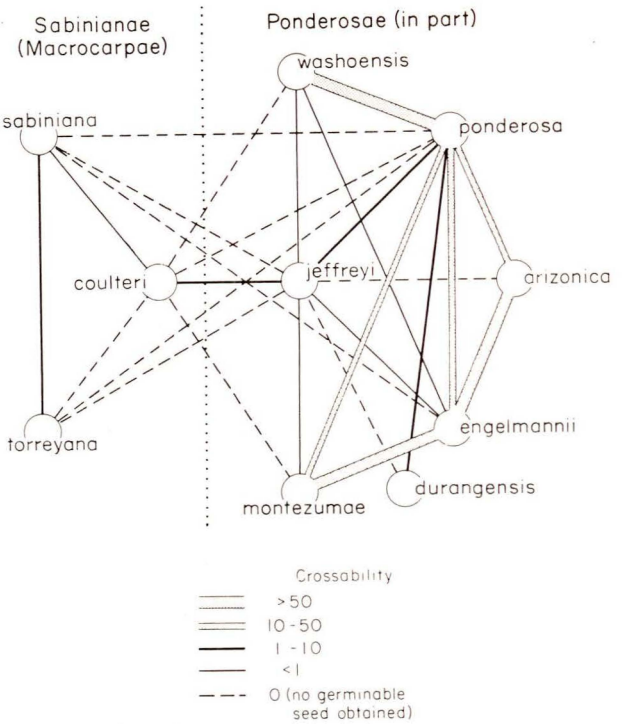


FIGURE 1. — Crossability of the Sabinianae and Ponderosae pines.

and 0.93 in Coulter pine, compared to an average of 0.55 for Jeffrey pine (Zobel 1953; Griffin 1964).

The distinctness of the Sabinianae and the species included in it suggests that this may be a very old group. The restricted ranges of these pines, especially the very limited and discontinuous range of Torrey pine, lend some support to this suggestion, and so does the extreme genetic isolation of these species, compared, for example to the Ponderosae (fig. 1) or the southern pines (Critchfield 1963). The relative uniformity of Digger and Coulter pines (Griffin 1964; Zobel 1953) also suggests that these are old species. The fossil record of the group is not extensive, but cone scales and seeds of pines not unlike the present-day Sabinianae have been found in Pliocene deposits in southern California (Dorf 1933; Axelrod 1937).

Although there are good morphological and other grounds for associating Jeffrey pine with the Ponderosae, it should be emphasized that this species occupies a unique and isolated position in the Ponderosae. It is separated by strong reproductive barriers from the rest of the group, in marked contrast to the relative ease with which most of the other species of the Ponderosae can be crossed with each other (fig. 1).

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# A NEW SPRUCE HYBRID - PICEA SCHRENKIANA X P. GLAUCA


**by D. P. Fowler**[1]


*Picea schrenkiana* Fisch and Meyer is a relatively unknown central Asiatic species confined to the Ala Tau and Thian Shan mountains of Turkestan. Morphologically the species is most closely related to *P. spinulosa* (Griff.) Henry and, to a lesser degree, to *P. obovata* Ledeb. (Wright 1955).

The genetic relationship between *P. schrenkiana* and other spruce species is unknown. A review of the literature failed to turn up any attempts to produce interspecific hybrids with this species. No reports of natural hybrids with *P. schrenkiana* were found.

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## Materials and Methods

In the spring of 1947, six *P. schrenkiana* seedlings were obtained from the nursery of Hillier and Sons, Winchester, England. These were planted as specimen trees at the Southern Research Station, Maple, Ontario. Subsequently one seedling died. The remaining five specimens are winter hardy but relatively slow growing (largest tree 17 feet at 18 years).

All five trees produced male and female strobili for the first time in 1964. The female strobili were isolated when they were still partially enclosed by bud scales and before the strobili scales had begun to separate (see Nienstaedt 1958). The isolation

technique was similar to that used by Mergen, Rossol, and Pomeroy (1955) for southern pines except that the sausage casing bags were coated on one side with aluminum paint. The painted side of the bag was positioned so that it shaded the strobili and enclosed foliage during mid-day.

Pollen of *P. omorika* (Pancic) Purkyne (1 tree), *P. glehnii* (Fr. Schmidt) Masters. (3 trees), and *P. jezoensis* (Sieb. and Zucc.) Carr. (1 tree) was obtained from trees located in the Durand-Eastman Park, Rochester, N.Y. In addition, pollen of *P. koyamai* Shirasawa (6 trees), *P. mariana* (Mill.) B.S.P. (10 trees), and *P. glauca* (Moench) Voss. (10 trees) was collected from trees growing at the Southern Research Station. Pollen was also collected from each of the five *P. schrenkiana* trees. In each instance, male strobili were collected just prior to natural pollen shedding and dried in paper boxes. The pollen was extracted by passing it through a 60-mesh Tylor screen.

Pollinations were made, using an aspirator type device, when the scales of the female strobili were well separated. A list of the attempted pollinations is given in table 1. The isolation bags were removed after the cone scales had closed. Fiberglas bags were placed over the cones in August and the cones were allowed to ripen on the trees. The ripe cones were collected in October, the seed extracted, and the full and empty seeds were separated by flotation in absolute alcohol. The full seeds were stratified for 30 days at 35-40°F on moist sand in Petri dishes, and germinated at 80°F in the laboratory. The germinated seeds were transplanted into individual plastic pots in a greenhouse, where they were raised under an 18-hour photoperiod with a 60°F night temperature and a 75-80°F day temperature.

One week after planting in the greenhouse, the seedlings of one population of *P. schrenkiana* x *P. glauca* were arranged in a test where they could be compared with three *P. glauca* and two *P. schrenkiana* populations. The three *P. glauca* populations resulted from controlled cross-pollinations of three of the trees used as male parents of the hybrids. The two *P. schrenkiana* populations resulted from self- and open-pollination of the female parent of the hybrids. The test consisted of a random row design (13 seedlings per row) replicated five times for a total of 65 seedlings from each of the six populations.

At approximately 6 weeks of age, all the seedlings were examined and the following observations were recorded: hypocotyl color, cotyledon shape, and cotyledon length. Munsell Color Charts

Table 1.--Summary of pollination results

| FEMALE PARENT PICEA SCHRENKIANA | MALE PARENT | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | OPEN | SELF | P. GLAUCA (10)[1] | P. MARIANA (10)[1] | P. OMORIKA (1)[1] | P. GLEHNII (3)[1] | P. JEZOENSIS (1)[1] | P. KOYAMAI (6)[1] |
| S 32 | | 1<br>42.0<br>100.0<br>42 | 1<br>2.0<br>100.0<br>2 | 2<br>0.0<br>—<br>— | 2<br>0.0<br>—<br>— | | | |
| S 33 | 3<br>14.0<br>95.2<br>40 | 12<br>29.2<br>99.0[2]<br>198 | 12<br>0.9<br>90.9<br>10 | 12<br>0.0<br>—<br>— | 12<br>0.0<br>—<br>— | 12<br>0.2<br>0.0<br>— | 12<br>0.0<br>—<br>— | 9<br>0.0<br>—<br>— |
| S 34 | | 6<br>39.8<br>98.5<br>197[2] | 7<br>0.1<br>100.0<br>1 | 3<br>0.0<br>—<br>— | 4<br>0.0<br>—<br>— | 5<br>0.0<br>—<br>— | | 7<br>0.1<br>100.0<br>1 |
| S 35 | 62<br>31.2<br>89.3<br>268 | 16<br>41.0<br>95.0[2]<br>190 | 11<br>1.6<br>38.9<br>7 | 7<br>0.0<br>—<br>— | 5<br>0.0<br>—<br>— | 6<br>0.3<br>50.0<br>1 | 13<br>0.0<br>—<br>— | 13<br>0.0<br>—<br>— |
| S 36 | 53<br>57.1<br>92.3[3]<br>277 | 13<br>89.1<br>99.0[2]<br>198 | 16<br>27.6<br>94.8<br>418 | 14<br>0.0<br>—<br>— | 12<br>0.0<br>—<br>— | 15<br>0.0<br>—<br>— | 14<br>0.0<br>—<br>— | 14<br>0.0<br>—<br>— |

CONTROLLED POLLINATIONS THAT YIELDED VIABLE SEEDS

- No. CONES POLLINATED
- No. FULL SEEDS/CONE
- PERCENT GERMINATION
- No. SEEDLINGS AT 2 MO.

[1] No. MALE PARENTS USED
[2] 200 SEEDS GERMINATED
[3] 300 SEEDS GERMINATED

(Munsell Color Co. 1952) were used to score hypocotyl color. When the seedlings were approximately 9 weeks old, total height and cotyledon number were recorded for the seedlings in the replicated test. Total height was again recorded for these seedlings at 15, 18, and 22 weeks.

### Pollination Results

The pollination results are presented in table 1. *P. mariana, P. omorika,* and *P. jezoensis* failed to produce any viable seeds when used as male parents on *P. schrenkiana. P. glehnii* and *P. koyamai* produced only one viable seed each when used as male parents. The authenticity of these hybrids is questionable. Of the six interspecific crosses attempted, only the cross *P. schrenkiana* x *P. glauca* can be considered reasonably successful. All five trees, used as female parents, produced some viable seed following pollination with *P. glauca;* but the success of this cross differed markedly between the five parents. Only one of the five trees could be considered to be moderately cross-compatible (27.6 viable seeds per cone).

*P. schrenkiana* would appear to be a fairly self-fertile species. Self-pollination resulted in a higher percentage of viable seed per cone than open pollination. The low percentage of full seed in the open-pollinated cones probably was the result of inadequate pollination. Further tests are required before a valid comparison of self- and cross-pollination can be made.

### Description of Hybrid *P. schrenkiana* x *P. glauca*

Seedling hypocotyl color at 6 weeks of age was found to be a useful attribute for distinguishing hybrid seedlings from non-hybrid seedlings of the female parent. *P. schrenkiana* seedlings then had yellow hypocotyls (Munsell Color Chart 5.0Y 7/8), while the seedlings of *P. glauca* had red hypocotyls (Munsell Color Chart 2.5R 4/8). At 6 weeks the hybrids had red hypocotyls (Munsell Color Chart 2.5R 4/6) which faded to a yellowish yellow-red (7.5YR 6/6) when the seedlings were 8-10 weeks old. The hybrids were similar to the female parent in respect to cotyledon length and were intermediate between the two parents in respect to cotyledon shape. *P. schrenkiana* seedlings had strongly upcurved cotyledons, whereas those of *P. glauca* were almost horizontal and straight. The cotyledons of the hybrids were slightly curved upward. The terminal shoots of the *P. glauca* seedlings were strongly curved upward. The terminal shoots of the *P. glauca* seedlings were strongly orthotropic, while those of *P. schrenkiana* were plagiotropic. The hybrids were intermediate in respect to this attribute.

The seedling height data obtained from the replicated test were subjected to a variance analysis and to a Duncan Range test (Duncan, 1955). The summarized results are presented in figure 1. Figure 2 shows an average seedling from each of the six populations at 12 and 22 weeks of age.

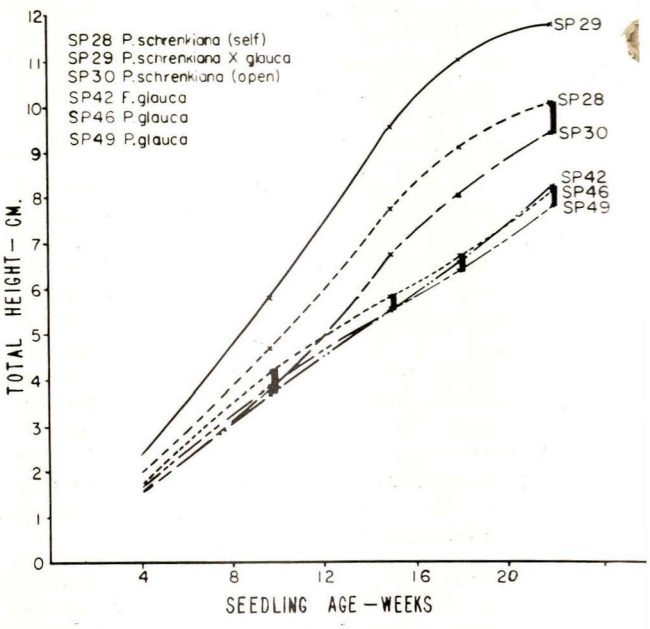


FIGURE 1. — Seedling height in relation to age. Any two points on the growth curves which are connected by the same vertical line are not significantly different at the 1-percent level.

The hybrids did not differ significantly from the female parent in respect to number of cotyledons. They were clearly superior to either parent species in respect to total height at 9, 15, 18 and 22 weeks. Under the conditions of this experiment, the hybrids exhibit heterosis.

### Relationship of *P. schrenkiana* to Other Spruce Species

The phylogenetic relationship between *P. schrenkiana* and the other spruces is obscure although it appears to be fairly closely related to *P. glauca. P. schrenkiana* is certainly an exception to the general pattern of crossability reported by Wright (1955) in that it is geographically and morphologically widely separated from *P. glauca.*

Wright (1955) considered *P. jezoensis* to be the most probable connecting link between the Old World and the western American spruces in that it is similar taxonomically to *P. sitchensis* (Bong.) Carr. and *P. engelmanni* (Parry) Engelm., and crosses easily with *P. glauca.* If *P. jezoensis* were the connecting link between the Old World and the western American spruces, one would expect the cross *P. schrenkiana* x *jezoensis* to be more successful than *P. schrenkiana* x *glauca.* This was not found to be true in this experiment. *P. jezoensis* failed to produce any viable seed when used as male parent with *P. schrenkiana.* As pollen from only one *P. jezoensis* was used on only three *P. schrenkiana* trees, it is still quite conceivable that the two species can be crossed (see Langner 1959).

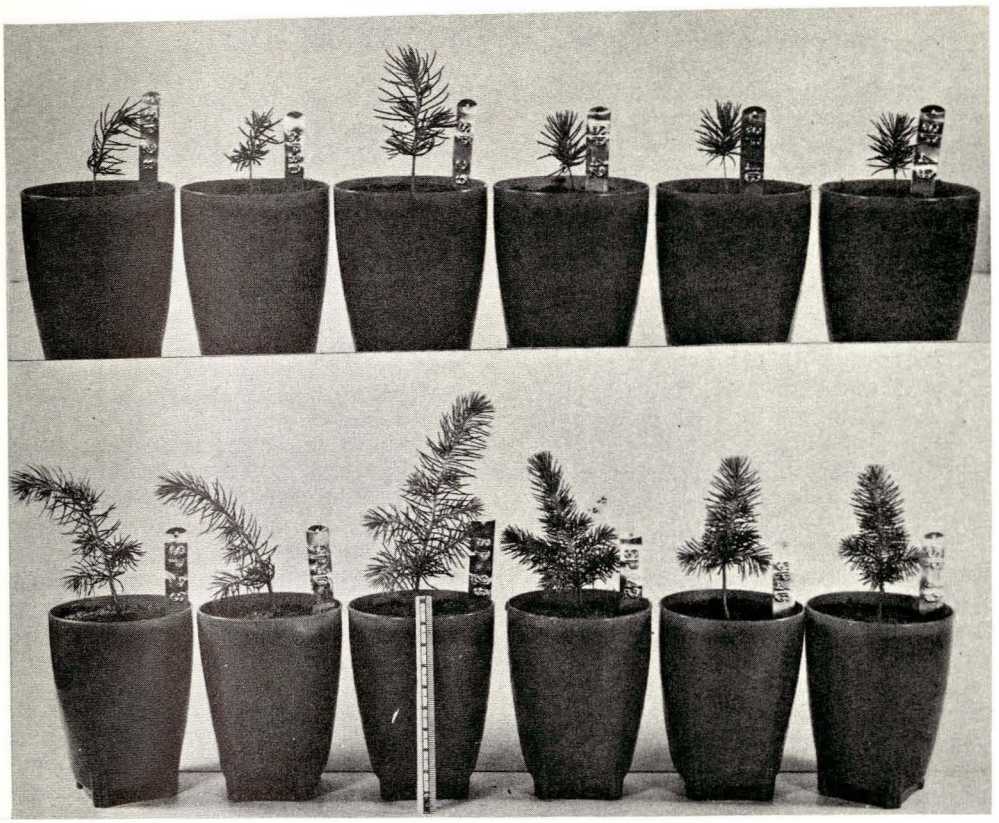

FIGURE 2. — Average seedlings at 12 (top) and 22 (bottom) weeks of age. Parentage from left to right: *P. schrenkiana* (self), *P. schrenkiana* (open), *P. schrenkiana* x *glauca*, *P. glauca*, *P. glauca*, and *P. glauca*.

In addition, the cross *P. glauca* x *P. koyamai* is reasonably successful (Wright 1955) and yet in this study the cross *P. schrenkiana* x *P. koyamai* was unsuccessful.

The preceding suggests that *P. glauca* and not *P. jezoensis* is the connecting link between the Old World and the western American spruces.

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# STUDIES OF COMPATIBILITY IN BETULA

by Knud E. Clausen[1]

Heterozygosity appears necessary to maintain fitness in many plant populations, and mechanisms favoring or enforcing cross pollination have evolved. Most important among the latter are dioecism and incompatibility.

While dioecism is comparatively rare in plants, incompatibility is common (Lewis 1954) and may be an important barrier to hybridization attempts. Knowledge of the amount of self-, intraspecific, and interspecific compatibility in a genus permits effective planning of a breeding program. Information on interspecific compatibility may also elucidate phylogeny and evolution within a genus.

Compatibility in the genus *Betula* is being studied at the Institute of Forest Genetics, Rhinelander, Wis. Most crosses have been made on grafts in the greenhouse, but data from some field crosses are included. An average of 5 to 6 female inflorescenes (catkins) were pollinated in each cross. The number of ovules per female catkin varies with the species but ranges from about 200 to 400. The work is continuing, and only the results of the first 3 years of tests are presented here.

## Self-Compatibility

Self-compatibility was studied in 31 individual birches belonging to nine species (table 1). No catkins or seed developed after 12 pollinations, while 6 crosses resulted in catkins containing inviable seed. Viable seed was produced from 15 selfings, but the germination percentages varied greatly. The two types of unsuccessful crosses may represent different degrees of incompatibility. The female inflorescence would be expected to abort if the pollen were completely inhibited and failed to germinate. Pollen germination, on the other hand, may permit the catkin and ovules to develop normally although the pollen tubes are inhibited before fertilization is effected.

Selfing of *Betula lenta* L. and *B. pumila* L. was unsuccessful, but must be repeated with more individuals before anything can be said about self-compatibility of these species in general. Three native species, *B. glandulosa* Michx., *B. nigra* L., and *B. populifolia* Marsh. appear to be nearly self-incompatible. Only a few crosses succeeded, and the germination was 2 percent or less in all cases (table 1).

Three out of four *B. papyrifera* Marsh. trees set seed which ranged from 6 to 11 percent germination. A certain amount of self-compatibility thus appears to be present in this species. The five trees of *B. alleghaniensis* Britt. tested so far have all been partially self-compatible but have varied considerably in the amount of filled seed produced (table 1). Variation in reaction from year to year has also been apparent. The tree which failed to set seed after selfing in 1963 had the highest germination percentage encountered so far, 45 percent, in 1964. This shows the importance of re-testing individuals which may appear self-incompatible in the first trial.

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Table 1.--Results of self-pollinations in Betula

| Species | No. of plants tested | Number of crosses with- No seed | Inviable seed | Viable seed | Percent germination of successful crosses |
|---|---|---|---|---|---|
| B. alleghaniensis | 5 | 1 | - | 5 | 0.5-5-10-16-45 |
| B. glandulosa | 3 | 1 | - | 2 | < 1-1 |
| B. lenta | 1 | 1 | - | - | |
| B. nigra | 5 | 3 | 1 | 1 | < 1 |
| B. papyrifera | 4 | 1 | - | 3 | 6-18-11 |
| B. pendula | 10 | 4 | 5 | 2 | < 1-< 1 |
| B. populifolia | 1 | - | - | 1 | 2 |
| B. pubescens | 1 | - | - | 1 | 0.5 |
| B. pumila | 1 | 1 | - | - | |
| Total | 31 | 12 | 6 | 15 | |

Two exotic species, both European, have been included in the tests to date. Trees of *B. pendula* Roth (*B. verrucosa* Ehrh.) have mostly been self-incompatible with only 2 out of 10 trees setting seed and these had less than 1 percent germination. The single *B. pubescens* Ehrh. tree tested was nearly self-incompatible.

## Intraspecific Compatibility

Five species and 37 individual trees of *Betula* have been used in the intraspecific tests so far (table 2). In the native species, 13 of 15 attempted crosses were successful, while one cross each of *B. alleghaniensis* and *B. papyrifera* failed to set seed. In both cases the pollen used was fertile when used in other crosses, indicating that the trees considered here probably are incompatible. The two *B. papyrifera* trees are growing on a small island and could conceivably be related. The germination percentages of the successful crosses have been good to excellent in the four native species, with crosses of *B. nigra* consistently best, followed by *B. papyrifera, B. alleghaniensis* and *B. lenta* crosses (table 2).

Six of the *B. pendula* crosses failed to produce catkins, 18 set inviable seed, and 13 were successful (table 2). Most of these crosses were between "normal" trees and trees with "curly" wood or varieties with cut or colored leaves. Sterile pollen may account for the lack of seed in one cross and the inviable seed in five crosses since the pollens were nonfunctional in other combinations as well. The remaining 18 unsuccessful crosses were all made with pollen that proved fertile in other crosses and must have failed for other reasons. Misjudgment of pistillate flower receptivity and environmental influences are possible causes. Several trees are located in an area where frequent frosts in late spring and early fall could have damaged the flowers.

Five of the 13 *B. pendula* crosses listed as successful had 1 percent or less germination and thus should probably be considered almost incompatible. Four of the successful crosses had between 11 and 23 percent germination, while four ranged from 39 to 81 percent.

## Interspecific Compatibility

Seven native and two European species have been included in tests of interspecific compatibility so far (table 3). No seed was produced in 44 of the attempted crosses, 50 set inviable seed, and 48 were successful. Of the crosses producing viable seed 40 had germination percentages ranging from less than 1 to 5 percent, indicating a high degree of incompatibility in these crosses. Only 8 crosses displayed fair to good compatibility, with germination percentages between 18 and 79 percent.

Interspecific crosses with *B. nigra* as either female or male parent have been difficult to make (table 3), and only a few seedlings have resulted from the successful crosses. Relatively few crosses involving *B. lenta* have been attempted to date and only the *B. lenta* x *alleghaniensis* cross had high germination. However, all seedlings raised from this cross died within a few months. Whether this represents true "hybrid break-down" or had other causes is unknown. *Betula alleghaniensis* has crossed well with *B. papyrifera* in both directions but poorly with other species. Of the *B. pendula* crosses only *B. pendula* x *papyrifera* and its reciprocal have been very successful. Most crosses with *B. populifolia* have produced few viable seeds and small seedling populations. Except for one combination where it was used as the male parent *B. pubescens* has performed similarly. Successful combinations involving *B. papyrifera* have already been mentioned. As the female parent *B. glandulosa* has been successfully crossed with *B. pubescens*

Table 2.--Results of intraspecific Betula crosses

| Species | Number of crosses with- | | | Percent germination of successful crosses |
|---|---|---|---|---|
| | No seed | Inviable seed | Viable seed | |
| B. alleghaniensis | 1 | -- | 3 | 31-47-51 |
| B. lenta | - | -- | 1 | 36 |
| B. nigra | - | -- | 5 | 63-77-96-96-100 |
| B. papyrifera | 1 | -- | 4 | 21-65-67-95 |
| B. pendula 1/ | 6 | 18 | 13 | From < 1 to 81 |
| Total | 8 | 18 | 26 | |

1/ Including 4 varieties.

only, and in a few attempts as the male parent it has crossed with difficulty. Crosses with *B. pumila* have largely been unsuccessful, but the attempts are too few to be meaningful.

## Discussion

The present study shows that self-incompatibility is common in species of *Betula,* but the degree of incompatibility varies with the individual plants. The results agree with previous work in Europe. Selfing of *B. pendula* produced an average of 2.6 percent filled seed in Finland (Hagman 1963) and from 0 to 60 percent viable seed in Germany (Stern 1963c). Selfed Finnish *B. pubescens* yielded 0.25 percent filled seed (Hagman 1963).

Self-fertile trees are probably undesirable in a breeding program or seed orchard. On the other hand, the high degree of self-compatibility present in certain trees makes it possible to use selfing as a means of approaching homozygosity.

The results of the intraspecific crosses show that while most *Betula* trees can be crossed without difficulty, some incompatibility occurs. Apparent incompatibility in the *B. pendula* crosses reported here was unusually high, possibly in part due to inbreeding. Three parent trees in four of these crosses came from the same Swedish seedlot and could conceivably be half-sibs. Similarly, seven other trees which proved incompatible or nearly so when intercrossed are of a single Finnish origin. That crosses between different seed sources of *B. pendula* can be succesful has been shown by Vaclav (1961), who used pollen from the U.S.S.R., Poland, Germany, France, and Belgium on Czechoslovakian trees with excellent results.

Although comparatively little work has been done on intraspecific compatibility in birch, the results obtained so far should emphasize the importance of testing all trees to be used in basic studies, breeding programs, or seed orchards.

Reproductive isolation of species is caused by a number of different mechanisms, including incompatibility. That reproductive isolation between species of *Betula* is not complete is evident both from the number of natural interspecific hybrids reported in the literature and from the results of artificial hybridization.

Table 3.--Number of attempted and successful interspecific Betula crosses and best results obtained [1/]

| Female Series [2/] | Female Species [3/] & ploidy | Male Series | Male Species & ploidy | nig 2X | len 2X | all 6X | pen 2X | ppf 2X | pub 4X | pap 4-6X | gld 2X | pum 4X |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Series [2/] Costatae | Costatae | Costatae | Albae | Albae | Albae | Albae | Nanae | Nanae |
| | | | | Species [3/] and ploidy | | | | | | | | |
| Costatae | nig | 2X | | | 1/0<br>0 | 5/1<br>1 | 7/5<br>2.5 | | 1/0<br>0 | 8/0<br>0 | | |
| Costatae | len | 2X | | 2/0<br>f | | 1/1<br>41 [4/] | 1/0<br>f | | 1/0<br>f | 2/0<br>0 | | |
| Costatae | all | 6X | | 9/1<br>1 | 2/0<br>0 | | 4/0<br>0 | | 1/1<br>1 | 8/4<br>79 | | |
| Albae | pen | 2X | | 6/1<br>< 1 | | 3/1<br>< 1 | | 2/0<br>0 | 3/0<br>0 | 7/4<br>18 | | 1/0<br>0 |
| Albae | ppf | 2X | | 1/1<br>< 1 | | 1/1<br>< 1 | 1/1<br>3 | | 1/1<br>1 | 1/1<br>3 | 1/1<br><1 | 1/1<br>3 |
| Albae | pub | 4X | | 1/1<br>1 | | 1/1<br>< 1 | 1/1<br>< 1 | 1/0<br>0 | | 1/1<br>1 | 2/1<br>1 | 1/1<br>< 1 |
| Albae | pap | 4-6X | | 7/5<br>1 | 3/1<br>< 1 | 7/5<br>23 | 4/3<br>58 | 1/0<br>f | 1/0<br>0 | | 1/0<br>f | 1/0<br>f |
| Nanae | gld | 2X | | 4/0<br>0 | | 3/0<br>0 | 3/0<br>0 | 3/0<br>0 | 3/2<br>60 | 3/0<br>f | | 1/0<br>f |
| Nanae | pum | 4X | | 2/0<br>f | | 1/0<br>f | 1/0<br>f | 1/0<br>f | 1/0<br>f | 1/0<br>f | | |

1/ In each box of the data columns, the first figure shows the number of attempts, the second shows number of crosses producing viable seed, and the third (beneath the second) shows highest percent germination (f = no seed).

2/ According to Winkler (1904).

3/ Key to Species: nig = nigra; len = lenta; all = alleghaniensis; pen = pendula; ppf = populifolia; pub = pubescens; pap = papyrifera; gld = glandulosa; pum = pumila

4/ Progeny inviable.

Variation in germination percentages occurred even within apparently successful species combinations in table 3. This can be attributed mainly to differences in compatibility between the individual plants used. European work with *B. pendula* x *pubescens* and its reciprocal cross has also demonstrated large differences due to individual trees (Eifler 1960; Vaclav 1961; Hagman 1963; Stern 1963a).

The demonstrated compatibility between *B. pendula* and *B. papyrifera* agrees with findings by Johnsson (1945) in Sweden, Klaehn (1952) in Germany, and Vaclav (1961) in Czechoslovakia. The last author reported better compatibility between *B. pendula* and *B. alleghaniensis* than found so far in this study. That *B. pubescens* crosses with other species only with difficulty, particularly when used as the female parent, has also been the experience in Europe (Johnsson 1945; Klaehn 1952; Vaclav 1956; Eifler 1960).

What are the possible causes of incompatibility and how can it be overcome? Incompatibility in *B. pendula* and *B. pubescens* is expressed by retarded growth of the pollen tubes in the styles (Hagman 1963). Tube growth inhibition is apparently due to an enzymatic reaction and seems to be specific, i.e., only self pollen or pollen of another species is inhibited. Thus, when Hagman (1963) pollinated with a mixture of self pollen and other pollen of *B. pendula,* the presence of self pollen did not reduce the seed set. Similar results were obtained when a mixture of pollen from two species was used (Vaclav 1961; Hagman 1963). Since enzymatic reactions are temperature-sensitive, changes in temperature may partially overcome the incompatibility. Low temperatures during pollen germination increased seed set after selfing of *B. pendula* and *B. pubescens* (Hagman 1963; Stern 1963c). High temperatures, on the other hand, have been effective in other plants (Townsend 1965; Kwack 1965). Pollination at late stages of female flower development has increased self-compatibility in *B. pendula* and *B. pubescens* and has also been effective in crosses between these species (Hagman 1963).

The incompatibility reaction in many plants is controlled by a single gene, *S*, which usually has many alleles. Stern (1963c) found evidence that this gene may be present in *B. pendula,* and it may occur in other species also.

In the gametophytic system of incompatibility the effect of the *S* gene may be overcome by polyploidy. Doubling the number of chromosomes results in the pollen carrying two different S alleles and may lead to complete self-compatibility (Lewis 1956). Irradiation offers another solution to overcoming incompatibilities. Fruit set in *Prunus avium* L. has been increased by treating the pollen mother cells with X-rays (Lewis, 1954). Apparently the irradiation caused the *S* gene to mutate to a self-compatibility allele. This treatment may also break crossing barriers between certain species, since the two incompatibility systems appear to be related and may be controlled by the same gene (Lewis 1956; Stern 1963b).

Within-series crosses might be expected to exhibit higher compatibility than between-series crosses. There is some evidence in the literature (Johnsson 1945; Klaehn 1952) and in table 3 that this may be true, particularly in crosses within the series *Albae.* However, it is also apparent in table 3 that certain between-series crosses can be as successful as crosses within series. The poor crossability shown by *B. nigra* may indicate that this species is only distantly related to the other species considered here. The fact that it also differs from the other native species in other respects, e.g. natural range, habitat, time of seed maturation, agrees with this suggestion.

European work and the present study have shown that there are often large differences between reciprocal crosses (table 3). This may in part depend on the individuals used. For example, the result of crossing one *B. alleghaniensis* as female parent with a certain *B. papyrifera* was 79 percent germination, while the reciprocal cross gave less than 1 percent germination. But the same *B. papyrifera* produced 13 percent viable seed when crossed as female with another *B. alleghaniensis* tree. Similarly, one *B. glandulosa* (female) crossed with a certain *B. pubescens* (male) gave 60 percent germination while the reciprocal cross produced no viable seed. Only 1 percent sound seed resulted from a cross of the same *B. pubescens* with a different *B. glandulosa.*

Differences between species in chromosome numbers may complicate interspecific compatibility patterns in *Betula.* In *Solanum,* crosses between parents of similar ploidy show increased compatibility with increasing ploidy, and crosses between parents of dissimilar ploidy are most successful when the male parent has the higher chromosome number (Marks 1965). Insufficient information is available for *Betula* to determine whether compatibility increases with increasing ploidy levels. There are, however, indications that crosses succeed more readily when made in the direction low ploidy female x high ploidy male. European workers have consistently found the cross *B. pendula* (2X) x *pubescens* (4X) easier to make than the reciprocal cross (Johnsson 1945; Eifler 1958, 1960; Hagman 1963; Stern 1963a). In this study there are two combinations in support of this hypothesis and one against. The crosses *B. glandulosa* (2X) x *pubescens* (4X) and *B. lenta* (2X) x *alleghaniensis* (6X) were compatible while the reciprocals were practically incompatible. On the other hand, *B. papyrifera* (4-6X) x *pendula* (2X) has always been

more successful than the reciprocal cross (table 3). Although the results of crossing *B. alleghaniensis* with *B. papyrifera* vary according to the direction of the cross (table 3), they will not be considered in this connection due to uncertainty about the chromosome numbers of the *B. papyrifera* parents.

Most of the progenies raised from the interspecific crosses have been measured and scored for various characteristics and will be compared with appropriate control progenies in order to determine hybridity of the material. Analysis of the data is in progress.

Apomixis in *Betula* has been suggested by previous work in which seedlings were obtained from unpollinated controls in *B. alleghaniensis, B. lenta, B. papyrifera,* and *B. populifolia*[2], and *B. pendula* (Vaclav 1961). No such evidence was found in this study, although normal-appearing but inviable seed was produced from some of the unpollinated controls used throughout the study.

## Summary

Results after 3 years of tests show that self-compatibility in *Betula* usually is very low. Of the 31 individuals belonging to 9 species tested so far, most have been self-incompatible or have had 1 percent or less germination. However, occasional trees had higher degrees of self-compatibility, indicating some variation between trees in this characteristic. The best germination percentages have been 45 percent in *B. alleghaniensis* and 11 percent in *B. papyrifera.*

Intraspecific tests have varied from complete failure to 100 percent germination, depending on the individuals used in the crosses. Most of the crosses with native species were successful. *Betula nigra* crosses were consistently best, followed by *B. papyrifera,* and *B. alleghaniensis.* The results of the *B. pendula* crosses were highly variable, and only part of the failures could be ascribed to pollen inviability, technical problems, or unfavorable environment.

The results of the interspecific crosses varied greatly with only one-third of 142 attempts setting viable seed. *Betula nigra* appears difficult to cross with other species, while *B. alleghaniensis* has crossed well with *B. papyrifera* but poorly with other species. Other successful crosses include *B. papyrifera* x *pendula,* its reciprocal, and *B. glandulosa* x *pubescens.*

Crosses between series of the genus appear to succeed as readily as within-series crosses. There are often large differences between reciprocal crosses. This may partly depend on the individual parents and partly be due to differences between the species in chromosome numbers. Interspecific crosses between species of dissimilar ploidy seem to be more successful when the male parent has the higher chromosome number.

No evidence of apomixis in *Betula* was found in this study.

[2]*E. J. Schreiner, 1961. Personal communication concerning work done at the Northeastern Forest Experiment Station, Upper Darby, Penn., in 1939-1940.*

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# EVIDENCES OF THE INHERITANCE OF TURPENTINE COMPOSITION IN SLASH PINE

by A. E. Squillace and Gordon S. Fisher[1]

Within-species variation in turpentine composition has promise of great utility in pine genetics. Most of the early work on turpentine composition dealt with species differences, and the utility of such variation in the taxonomy of pines is well known (Mirov 1961). With the development of gas chromatographic techniques, intensive study of individual tree differences has become possible (Bannister *et al.* 1959; Williams and Bannister 1962); and this type of variation has been shown to occur in several pine species (Bannister *et al.* 1962; Blight and McDonald 1964; Smith, 1964a).

This paper shows that individual tree differences and racial variation occur in slash pine (*Pinus elliottii* Engelm.) and that differences in the content of some constituents are strongly inherited. Although further exploratory work is needed, the results reveal opportunities to breed for specific composition, to capitalize on geographic variation in commercial gum operations, and to develop a useful tool in pine genetics research.

## Methods

Oleoresin samples were taken from 174 trees as outlined below.

1. *Thirty-one 16-year-old rooted cuttings.* There were 15 clones, each containing from 1 to 5 ramets. Most of the ortets had been selected for either high or average gum yielding ability.
2. *Ten grafted trees.* These were members of two of the clones noted above. The grafts had been made on plantation saplings 7 years prior to sampling. There were 5 grafted trees per clone.
3. *Eleven selections.* These were mature trees growing in natural stands. Most had been selected for high gum yielding ability.
4. *Eighty progeny trees produced from matings among 9 of the ortets noted in "1" above.* They were included in 9 crosses, one self, and 3 wind-pollinations. There were from 4 to 14 trees per progeny. About half of the progenies were 16 years old, while the remainder, in a separate plantation, were 11 years old.
5. *Forty-two trees in 2 seed source tests.* There were 15 seed sources, with from 1 to 5 trees sampled per source. Most trees were 7 years old at time of sampling, while a few were 9 years old.

All trees sampled were growing within 15 miles of Olustee, Florida. Most oleoresin samples were taken in the fall of 1961, but some were taken at later dates.

Two kinds of oleoresin (gum) samples were taken, "cortex" and "stem." Cortex samples were obtained by excising branch terminals at about ½-inch from the tip. Samples were usually taken from branches in the lower crown. Usually, within a few minutes after cutting, a drop of gum exuded at the cut surface of the excised bud. This was removed with a spatula and inserted into a small vial. Vials were then sealed and stored in a refrigerator until analyzed. Samples collected in this manner consisted mostly, if not entirely, of gum exuding from resin ducts in cortical tissue.

Most stem gum samples were obtained by microchipping (Ostrom and True 1946) of the stem at about 2 feet above ground level. Storage was handled in the same manner as was done for cortex gum. Samples collected in this manner consisted mostly of gum exuding from resin ducts in secondary xylem tissues.

Chemical analyses were made by gas-liquid chromatography, and this was usually accomplished within 2 or 3 weeks after collection. Typical operating conditions were as follows: column, 10′ x ⅛″; support, Chromosorb-W 60-80 mesh; substrate, carbowax-20M; loading, 30 percent; column temperature, 120°C.; helium inlet pressure, 45 p.s.i.; sample size, 0.1 mg. whole gum dissolved in 3 volumes of acetone; injection temperature, 275°C. With this substrate, the column temperature is critical for optimum separation of myrcene from other components. Peak areas were determined with a Disc integrator and the composition was calculated on the basis of the total area through β-phellandrene. Only trace amounts of terpenes emerging after β-phellandrene were encountered. In standardization runs all the major terpenes gave approximately the same peak area per mg.

Since the data encompassed various field plots, trees of different ages, and several sampling dates, highly efficient statistical techniques were not possible. Simple analyses of variance and regression techniques were employed to the extent possible, but the results of these must necessarily be considered as approximations. Wherever required, estimates of ortet composition were obtained by averaging the clonal data.

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In presenting the results, percentages are shown for major constituents only ($\alpha$-pinene, $\beta$-pinene, myrcene, and $\beta$-phellandrene). Percentages of minor constituents, including camphene, limonene, and unidentified compounds, are lumped and shown as "other."

## Instrumental Reproducibility

Instrumental reproducibility was assessed by making 2 or 4 repeated injections of the same sample of cortex gum from each of 6 trees. Average composition and the average (pooled) standard deviation among determinations for the 6 trees are shown below.

| *Constituent* | *Average composition (percent)* | *Average standard deviation (percent)* |
|---|---|---|
| $\alpha$-pinene | 18.8 | .51 |
| $\beta$-pinene | 40.3 | 1.00 |
| Myrcene | 15.8 | .70 |
| $\beta$-phellandrene | 22.0 | .64 |
| Other | 3.1 | — |

The standard deviations were slightly higher than the average, 0.34 percent, reported by Bannister *et al.* (1962) for major constituents in Monterey pine (*P. radiata* D. Don). But reproducibility was considered sufficiently accurate to discontinue replicate determinations.

## Within-Tree Variation

Moderate correlations were found between the percentages of $\alpha$-pinene, $\beta$-pinene, and $\beta$-phellandrene in cortex and stem gum (table 1). However, turpentines in gum from these two sources differed markedly in several respects.

1. Cortex gum usually contained less $\alpha$-pinene and more $\beta$-pinene than stem gum.

2. Myrcene (and rarely, limonene) was present in appreciable quantities in the cortex gum of some trees but was very low in the stem gum of all trees sampled.

3. Trees with appreciable quantities of $\beta$-phellandrene in their stem gum invariably contained appreciable quantities in their cortex gum. However, the reverse was not always true. Some trees had high amounts of $\beta$-phellandrene in their cortex gum and low amounts in their stem gum. Usually the percentage of this constituent was higher in cortex gum than in stem gum.

Table 1.--The relation between cortex and stem gum extracted from 69 trees

| Constituent | Mean composition | | Correlation coefficient |
|---|---|---|---|
| | Cortex gum | Stem gum | |
| | Percent | Percent | |
| $\alpha$-pinene | 29.1 | 57.3 | .40** |
| $\beta$-pinene | 48.1 | 30.6 | .47** |
| Myrcene | 6.4 | 1.6 | (1/) |
| $\beta$-phellandrene | 14.1 | 9.0 | .56** |
| Other | 2.3 | 2.2 | (1/) |

1/ Not computed because of the low amounts of these constituents in one or both sources.
** Significant at the 1-percent level.

Gum in plant parts other than stem wood and branch terminals was not studied. In respect to differences in gum composition within tissues, Blight and McDonald (1964) and Smith (1964a) found only minor differences in samples of stem gum taken at various circumferential and vertical positions.

Repeated samples were taken on some trees on different dates, spanning a period of 3 years. Pooled standard deviations, representing differences between sampling dates, varied from about 2 to 9 percent for various constituents (table 2). $\beta$-pinene and $\beta$-phellandrene, both in cortex gum, showed the greatest variation. No clear seasonal pattern could be shown.

The causes of the differences in composition of gum collected at various dates are unknown. Blight and MacDonald (1964) and Smith (1964a) reported low seasonal variation for several pine species. In another publication, Smith (1964b) suggested that age effects on stemwood gum in ponderosa pine (*P. ponderosa* Laws.) are minor. Further study of seasonal and age effects is needed for slash pine.

Although the differences noted above seem large, they were relatively small in comparison to differences between trees.

## Individual Tree Variation

Large differences were found among trees in the composition of their turpentine in both cortex and stem gum, and evidence was obtained that much of the variation is genetic. Space prevents showing all of the data, but the results for 4 parent trees and 2 families sampled are given in table 3.

Note that parent trees G-1 and G-2 are unique in that they have a relatively low amount of $\alpha$-pinene in comparison to $\beta$-pinene and relatively large amounts of myrcene and $\beta$-phellandrene in their cortex gum. Six out of 8 of the progenies of the mating between these two parents closely resembled their parents. Two other parent trees, G-3 and G-4, are unique in having relatively high $\alpha$-pinene in comparison to $\beta$-pinene and containing only very small amounts of myrcene and $\beta$-phellandrene. Eight of the 9 progenies of G-3 x G-4 resembled their parents. Although the possibility of contamination cannot be excluded, the "maverick" individuals in the two families are probably due to control by few genes and heterozygosity in the parents. Mode of inheritance will be discussed later.

Table 2.--Variation in turpentine composition in samples taken from the same tree on different dates

(Percent)

| Item | α-pinene | β-pinene | Myrcene | β-phellandrene | Other |
|---|---|---|---|---|---|
| | | CORTEX GUM[1] | | | |
| Means | 25.8 | 41.5 | 8.1 | 22.7 | 1.9 |
| Standard deviations[2] | 3.4 | 6.3 | 3.9 | 9.4 | -- |
| | | STEM GUM[3] | | | |
| Means | 62.8 | 26.2 | 2.7 | 6.6 | 1.7 |
| Standard deviations[2] | 4.4 | 2.2 | 2.8 | 3.7 | -- |

1/ Based upon two samplings from each of 18 trees.

2/ Pooled "within-tree." The values are measures of the differences between percentages obtained from samples on the same tree but on different dates.

3/ Based upon two samplings from each of 9 trees.

Table 3.--A portion of the basic data, typifying the variation and inheritance of turpentine composition

(Percent)

| Mating | Tree No. | Cortex gum α-pinene | Cortex gum β-pinene | Cortex gum Myrcene | Cortex gum β-phellandrene | Cortex gum Other | Stem gum[1] α-pinene | Stem gum[1] β-pinene | Stem gum[1] Myrcene | Stem gum[1] β-phellandrene | Stem gum[1] Other |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | PARENTS | | | | | | |
| | G-1 | 12 | 36 | 17 | 32 | 3 | 44 | 30 | 1 | 23 | 2 |
| | G-2 | 16 | 52 | 17 | 13 | 2 | 44 | 40 | 1 | 12 | 3 |
| | G-3 | 40 | 56 | 1 | 2 | 1 | 80 | 17 | 1 | 1 | 1 |
| | G-4 | 38 | 59 | 1 | 1 | 1 | 62 | 35 | 1 | 1 | 1 |
| | | | | | PROGENIES | | | | | | |
| G-1 x G-2 | 1-5-5 | 13 | 48 | 19 | 18 | 2 | 60 | 25 | 1 | 10 | 4 |
| | 1-7-5 | 23 | 51 | 2 | 24 | 0 | 47 | 39 | 1 | 10 | 3 |
| | 1-10-6 | 10 | 27 | 32 | 28 | 3 | 47 | 29 | 1 | 20 | 3 |
| | 2-6-2 | 36 | 38 | 2 | 20 | 4 | 68 | 17 | 1 | 12 | 2 |
| | 3-6-5 | 13 | 32 | 20 | 31 | 4 | 70 | 16 | 1 | 11 | 2 |
| | 5-3-4 | 8 | 20 | 41 | 29 | 2 | 57 | 19 | 1 | 19 | 4 |
| | 7-10-7 | 12 | 29 | 20 | 36 | 3 | 58 | 15 | 1 | 22 | 4 |
| | 8-9-2 | 21 | 36 | 22 | 18 | 3 | 57 | 20 | 2 | 19 | 2 |
| G-3 x G-4 | 5-10-16 | 49 | 49 | 1 | 1 | 0 | 51 | 49 | 0 | 0 | 0 |
| | 10-1-5 | 11 | 45 | 22 | 20 | 2 | 54 | 24 | 1 | 18 | 3 |
| | 10-2-5 | 42 | 54 | 0 | 1 | 3 | 56 | 41 | 1 | 1 | 1 |
| | 10-3-5 | 49 | 48 | 1 | 1 | 1 | 84 | 15 | 0 | 0 | 1 |
| | 10-5-5 | 40 | 56 | 1 | 1 | 2 | 64 | 34 | 1 | 1 | 0 |
| | 10-6-5 | 51 | 40 | 2 | 1 | 6 | | | | | |
| | 10-7-5 | 38 | 58 | 1 | 1 | 2 | | | | | |
| | 10-8-5 | 53 | 40 | 2 | 1 | 4 | | | | | |
| | 10-9-5 | 42 | 50 | 1 | 2 | 5 | 65 | 28 | 1 | 1 | 5 |

1/ Trees 10-6-5, 10-7-5, and 10-8-5 were not sampled for stem gum.

Similar variation and inheritance can be seen for β-phellandrene in stem gum. In stem gum, however, myrcene is very low in all trees; also, inheritance is obscure for α-pinene and β-pinene in these data.

Analyses of variance were run on the progeny data, excluding the selfed progeny. Intraclass correlations, which show the percentage of the total variance that is associated with families, are shown in table 4. Heritability estimates were not made because of the nature of the data, but most constituents seem to be strongly inherited.

A part of the clonal data is shown in table 5. Note the similarity of ramets within clones. The

Table 4.--Intraclass correlations obtained from analyses of variance of progeny and clonal data

| Source of gum | α-pinene | β-pinene | Myrcene | β-phellandrene |
|---|---|---|---|---|
| | | PROGENIES | | |
| Cortex | .37** | .32** | .37** | .53** |
| Stem | .19* | .16* | -- | .58** |
| | | CLONES | | |
| Cortex | .95** | .96** | .95** | .95** |
| Stem | .88** | .79** | -- | .95* |

* Significant at the 5-percent level.
** Significant at the 1-percent level.

consistency within clones is especially remarkable in view of the fact that some of the ramets were rooted cuttings growing at one location while others were grafted trees growing in another location about 10 miles distant. In the grafted trees, the cortex gum samples were taken from branches above the graft unions. Neither the differences in location nor type of propagation had any appreciable effect upon turpentine composition.

Intraclass correlations for clonal data are shown in table 4. Once again, strong genetic variation is indicated. Note that intraclass correlations for $\alpha$-pinene and $\beta$-pinene were stronger in cortex gum than in stem gum. This was also true for progeny data.

Table 5.--A portion of the clonal data, typifying the variation and inheritance of turpentine composition

(Percent)

| Clone | Ramet 3/ | Cortex gum 1/ | | | | | Stem gum 2/ | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | α-pinene | β-pinene | Myrcene | β-phellandrene | Other | α-pinene | β-pinene | Myrcene | β-phellandrene | Other |
| G-1 | 1-3 | 16 | 36 | 18 | 28 | 2 | | | | | |
| | 2-3 | 13 | 34 | 22 | 28 | 3 | | | | | |
| | 4-3 | | | | | | 46 | 29 | 1 | 21 | 3 |
| | 5-3 | | | | | | 41 | 31 | 1 | 25 | 2 |
| | 6-3 | 13 | 32 | 23 | 29 | 3 | | | | | |
| | a | 10 | 39 | 15 | 33 | 3 | | | | | |
| | b | 10 | 40 | 14 | 33 | 3 | | | | | |
| | c | 11 | 37 | 14 | 36 | 2 | | | | | |
| | d | 11 | 37 | 15 | 36 | 1 | | | | | |
| | e | 10 | 37 | 15 | 36 | 2 | | | | | |
| G-4 | 2-7 | 38 | 57 | 2 | 2 | 1 | 60 | 37 | 1 | 1 | 1 |
| | 3-7 | 32 | 63 | 1 | 2 | 2 | 64 | 33 | 1 | 1 | 1 |
| | 6-7 | 33 | 62 | 2 | 2 | 1 | | | | | |
| | a | 43 | 55 | 1 | 1 | 0 | | | | | |
| | b | 39 | 58 | 1 | 1 | 1 | | | | | |
| | c | 37 | 61 | 1 | 1 | 0 | | | | | |
| | d | 39 | 59 | 1 | 1 | 0 | | | | | |
| | e | 39 | 57 | 1 | 1 | 2 | | | | | |

1/ Ramets 4-3 and 5-3 of clone G-1 were not sampled.
2/ Only the 4 trees indicated were sampled.
3/ Ramets labelled "a" through "e" were grafted trees in a plantation near Lulu, Florida. All other trees were rooted cuttings growing near Olustee, Florida.

Table 6.--Results of parent-progeny regression analyses

| Constituent | Cortex gum (Basis, 12 families) | | Stem gum (Basis, 10 families) | |
|---|---|---|---|---|
| | Regression coefficient | Coefficient of determination | Regression coefficient | Coefficient of determination |
| α-pinene | 1.04** | 0.83** | 0.23 | 0.11 |
| β-pinene | .35 | .12 | -.40 | .10 |
| Myrcene | .92** | .68** | -- | -- |
| β-phellandrene | .63* | .34* | .46 | .35 |

* Significant at the 5-percent level.
** Significant at the 1-percent level.

Parent-progeny regressions were run as a further check on inheritance of turpentine composition. Progeny means were used as the dependent variable. For crosses, the average of the female and male parent values was used as the independent variable. For wind-pollinated progenies the value for the female parent was used as the independent variable.

Regression coefficients for all constituents of cortex gum, excepting $\beta$-pinene, were strong (table 6). Those for $\beta$-pinene in cortex gum and all constituents of stem gum were weak (negative in the case of $\beta$-pinene).

## Mode of Inheritance

In order to obtain clues to the mode of inheritance, frequency distributions were compiled for each major constituent of turpentine. In compiling these, we omitted the seed source test data for Citrus, Volusia, and Polk Counties because many trees of these southern sources differed appreciably from the bulk of the data in the northern portion of the species range, a possible influence of south Florida slash pine (*P. elliottii* var. *densa* Little & Dorman)

The distributions for $\alpha$-pinene in both cortex and stem gum and for $\beta$-pinene in stem gum are quite normal, suggesting multigenic control for these compounds (figs. 1 and 2). The distribution for $\beta$-pinene in cortex gum is skewed to the left but is continuous and has a single mode. It is possible that the quantity of $\beta$-pinene in cortex gum is controlled by few genes but that non-genetic effects caused the continuous pattern.

The distributions for myrcene in cortex gum and $\beta$-phellandrene in both cortex and stem gum are definitely abnormal, being skewed and having a tendency toward bimodality. Approximately ¾ of the trees contained low amounts (0-4 percent) of myrcene, while about ¼ contained high amounts (10 percent or more) of this constituent. Control by few genes is suggested. The bimodality for $\beta$-phellandrene in both cortex and stem gum is not as clear as for myrcene, but nevertheless the distributions suggest control by few genes.

On the basis of these preliminary results, hypothetical models for the inheritance of myrcene in cortex gum and $\beta$-phellandrene in cortex and stem gum were postulated. However, the limited data available were not adequate for reliable test of the models. More progenies, especially selfs and other inbreds, are being sampled to test the hypotheses on mode of inheritance suggested for all major constituents. It is of interest to note that Forde (1964) postulated that the major difference between turpentines of knobcone pine (*P. attenuata* Lemm.) and Monterey pine is controlled by a single gene.

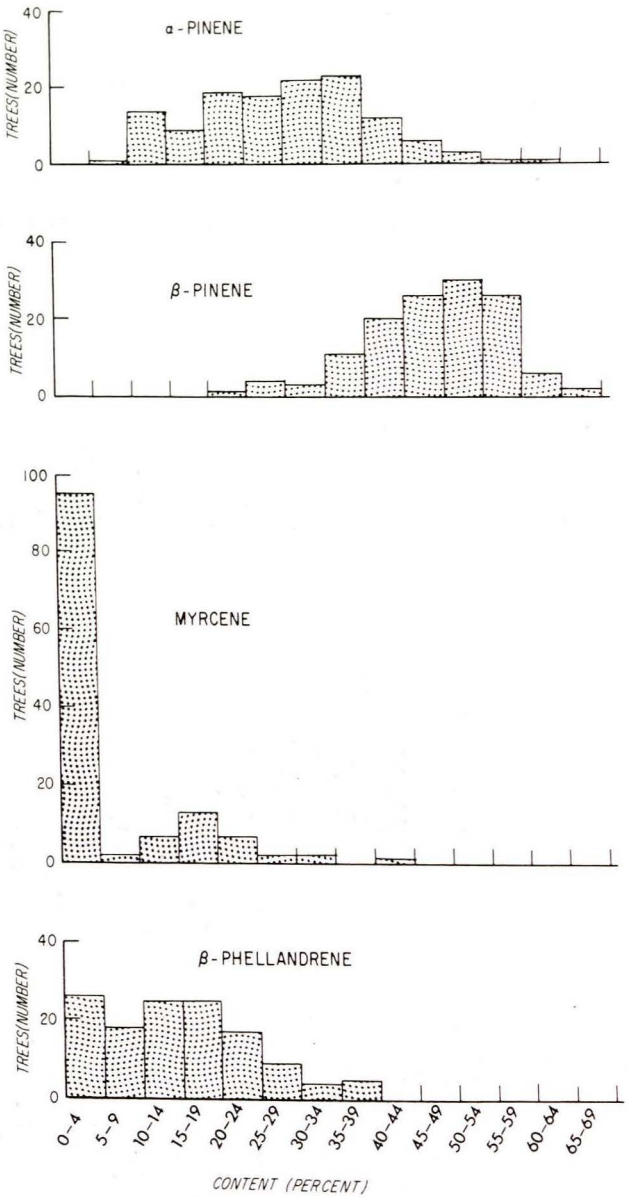


FIGURE 1. — Frequency distributions for the major constituents of turpentine in cortex gum. Basis, 129 trees.

## Evidences of Racial Variation

In order to study racial variation, all trees of known geographic origin other than progenies were utilized (the latter could not be used because most had parents of different origins). For cortex gum, 60 trees in 12 sources, with from 3 to 16 trees per source, were available for analysis. For stem gum, there were 26 trees in 5 sources, with from 5 to 6 trees per source. Analyses of variance were run, using a simple "between- and within source" model.

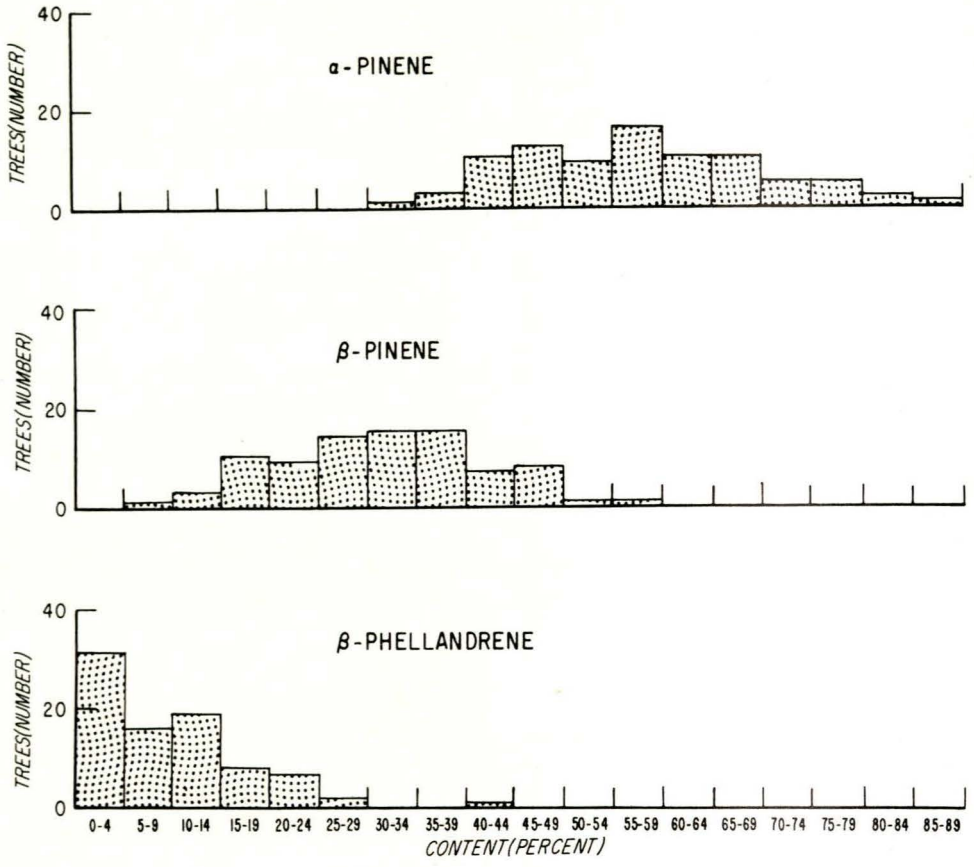


FIGURE 2. — Frequency distributions for the major constituents of turpentine in stem gum. Basis, 84 trees.

Seed source effects were significant or highly significant for all constituents except $\alpha$-pinene in both cortex and stem gum and for $\beta$-phellandrene in stem gum. $\beta$-pinene in cortex gum showed a clear pattern — content of this constituent was low in south Florida and increased northward, reaching a high in south Georgia, and then decreased near the northern limits of the species range (fig. 3). This pattern is similar to the total height pattern reported by Squillace and Kraus (1959) and also to patterns for several traits reported by Squillace (1964). $\beta$-phellandrene in cortex gum also showed a pattern, this constituent being low in the north and high in the south (fig. 4). Patterns for other constituents were either random or not clear. Because of the high variation among trees within sources and the few trees per source, these results are considered mainly as suggestive of further study.

Racial differences in turpentine composition have previously been reported for ponderosa pine (Mirov 1961), Monterey pine (Bannister *et al.* 1962) and bishop pine (*P. muricata* D. Don) (Forde and Blight 1964).

## Discussion and Conclusions

This exploratory study has given us an insight into the variation and inheritance of turpentine composition in cortex and stem gum of slash pine. The data showed that (1) composition of turpentine, in both sources of oleoresin, varies greatly among trees within stands and also among trees of different geographic sources; (2) the content of most constituents is strongly inherited; and (3) some constituents seem to be controlled mainly by a few genes, while others show polygenic inheritance.

The results suggest that we could easily breed for high or low amounts of some constituents, such as myrcene, $\alpha$-pinene, and $\beta$-pinene in cortex gum, and $\beta$-phellandrene in both cortex and stem gum. Unfortunately, the constituent presently considered to be most valuable, $\beta$-pinene in stem gum, seems to have the weakest genetic control. The racial differences suggest that we can control turpentine composition on a practical basis by concentration of gum extraction in certain areas or by keeping gum separate by geographic areas.

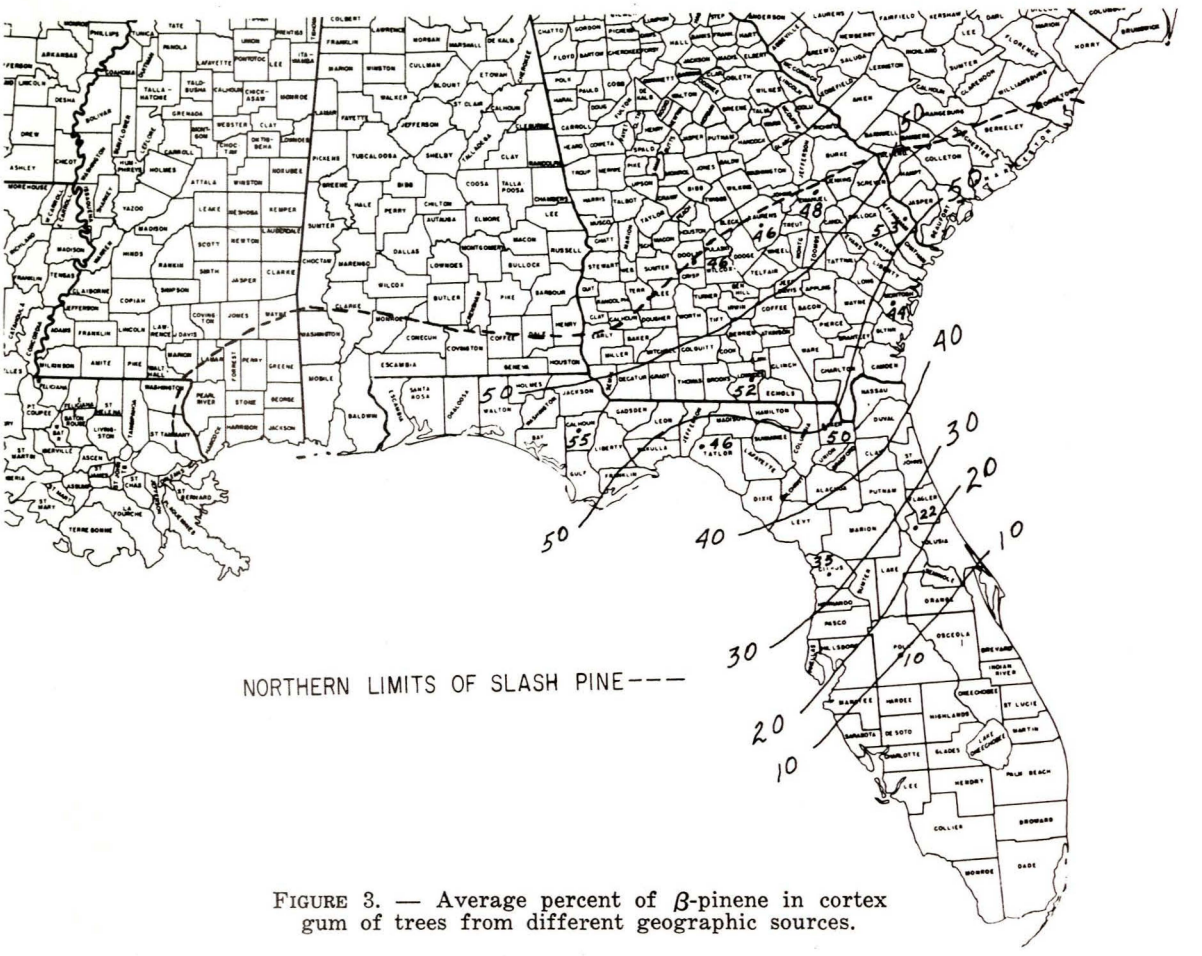


FIGURE 3. — Average percent of β-pinene in cortex gum of trees from different geographic sources.

Further study is needed, however, to verify the variation patterns and to determine more accurately the mode of inheritance of each constituent. Such knowledge will give us a better basis for judging the breeding potentials for each constituent. In addition, with precise knowledge of the mode of inheritance we may have a very useful tool for identifying relatives, hybrids, etc. The possibility of associations of differences in composition of both cortex gum and stem gum with insect resistance should be investigated.

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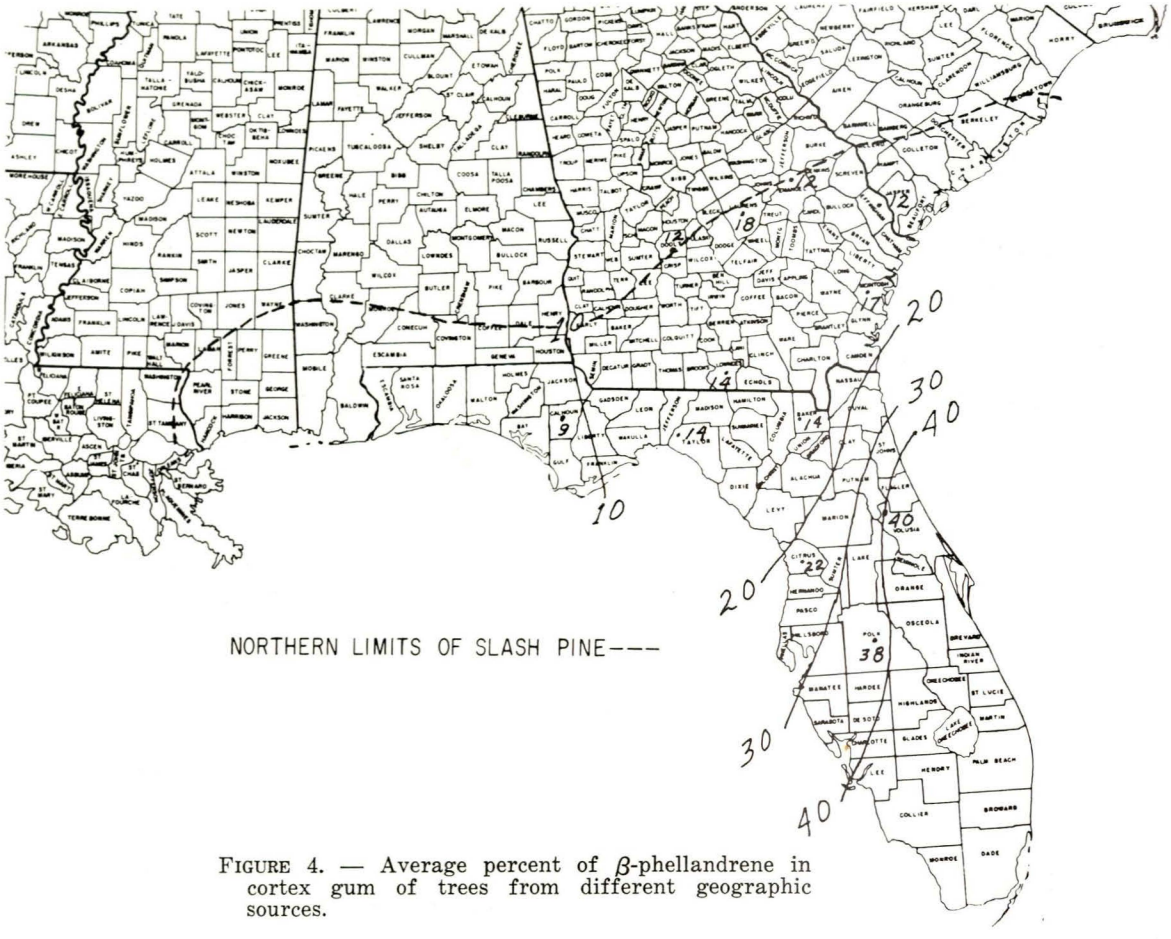


FIGURE 4. — Average percent of $\beta$-phellandrene in cortex gum of trees from different geographic sources.

# INHERITANCE OF GERMINATIVE ENERGY AND GERMINATIVE CAPACITY IN DOUGLAS-FIR

by Thomas E. Greathouse[1]

In the West foresters have had considerable difficulty in reforesting south-facing slopes. We considered this problem when we selected plus-trees for the first Douglas-fir seed orchard in Region 6. We were, however, faced with the need to answer such questions as these: (1) Should we try to produce seed inherently suited for south slopes? (2) If so, should we strive to get seed primarily of high germinative energy,[2] or is high germinative capacity[3] an adequate indication of suitability?

Before we can select for either of these traits we need to know how they are inherited. As part of a progeny test, therefore, we checked germinative energy and germinative capacity for the seeds from several control-pollinated full-sib Douglas-fir progenies.

## Procedure

The seeds developed on grafts in a seed orchard. The ortets are growing within 25 miles of each other on the Olympic National Forest in western Washington. The variation due to environmen-

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[2]*As used here, germinative energy is the number of days at which 50 percent of the total germination has been attained.*

[3]*As used here, germinative capacity is the percentage of the seeds sown that have germinated or are sound, but ungerminated at 49 days.*

tal differences between the sites in which the ortets are growing should, therefore, be relatively limited.

We planned to make a six-parent diallel cross excluding self pollinations. Some of the crosses, however, were not successful (table 1). To satisfy progeny test requirements we also used the same six clones as pollen parents in making crosses with several other clones. We plan to repeat this whole array of crosses in 1966 to verify the results reported in this paper.

Table 1.--Results of planned diallel crosses of six Douglas-fir clones[1/]

| Female parent tree no. | Male parent tree no. | | | | | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 7 | 8 | 22 | 23 |
| 1 | - | X | X | X | X | X |
| 2 | X | - | X | X | 0 | 0 |
| 7 | X | 0 | - | 0 | 0 | 0 |
| 8 | X | X | X | - | X | X |
| 22 | X | X | 0 | 0 | - | 0 |
| 23 | 0 | 0 | 0 | 0 | 0 | - |

1/ X = successful cross; - = no cross attempted; 0 = unsuccessful cross.

We pollinated the conelets in the spring of 1962. That fall we collected the cones, air-dried them, and extracted the seeds. We placed the seeds in paper envelopes which were then stored at 35° F. in a refrigerator from January to April 1, 1963.

The seeds were not stratified (but may have absorbed some moisture in the refrigerator) before we sowed them on April 1. At that time we took 150 filled seeds from each lot[4] and divided them into three weight classes (heavy, medium, and light).[5] We then divided the unequal number of seeds in the three weight classes into essentially equal groups and placed them on moist filter paper in petri dishes so that each petri dish contained 50 seeds (36 to 44 in three crosses), made up of one-third of the seeds from each weight class. On the same day we distributed the three petri dishes for each lot randomly in a growth chamber.

After an initial check on Friday and Saturday, April 5 and 6, we examined the petri dishes each Monday, Wednesday, and Friday for 7 weeks. When a seed produced a radicle about 3 millimeters long we tallied it as germinated. At the close of the test period we cut all ungerminated seeds and found no sound ones left.

[4]*Three crosses had fewer seeds: 106 for 1x2, 136 for 1x23, and 107 for 2x8.*

[5]*Classification was on the following weight basis: heavy, > 15.0 mg., medium, 13.0-15.0 mg., and light, < 13.0 mg.*

## Results

The germination test results brought out effects of both maternal and paternal parents on germination characteristics.

*Maternal influence.* — Curves of cumulative germination percent indicate what we interpreted to be a strong maternal influence. For example, seeds from clone No. 10 show high germinative energy, regardless of pollen parent (fig. 1). Furthermore they reach 50 percent of total germination about 7 days before seeds from clone No. 1 do. Unfortunately we made no crosses with clone No. 10 as a pollen parent.

Further evidence of the maternal effect was based on seeds produced when we applied pollen from clones Nos. 1 and 2 on conelets of clones Nos. 8, 10, and 22. We punched the data on IBM cards and used a program to give Student's "t" values (fig. 2). All three female parents had germinative energies that differed significantly at the 1-percent level.

*Paternal influence.* — The curves bring out a tendency for decreasing germinative capacity when clones Nos. 2, 22, 7, and 23 were used as pollen parents on clones Nos. 1, 10, and 8 (figs. 1 and 3). In addition, clones Nos. 10, 22, and 8 all produced seed with a higher germinative capacity when fertilized with pollen from clone No. 2 than when fertilized with pollen from clone No. 1 (fig. 2).

## Discussion

High germinative capacity is always a desirable attribute of seed. The fact, however, that Douglas-fir female parents may exert a strong influence on germinative energy of the seed, as indicated by

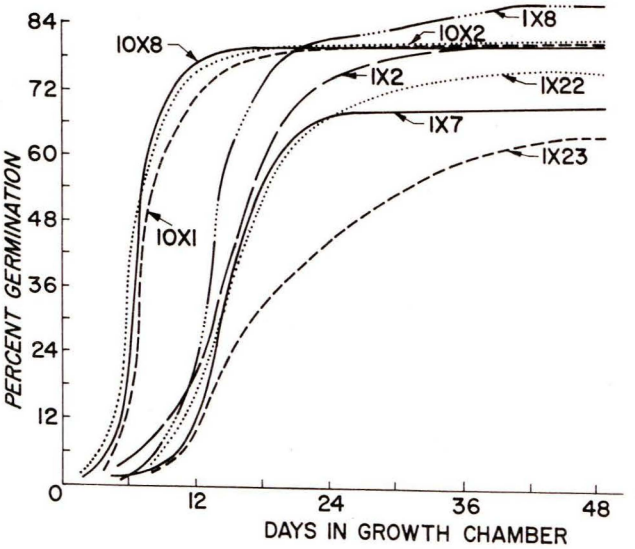


FIGURE 1. — Maternal influence of clones 1 and 10 on germinative energy.

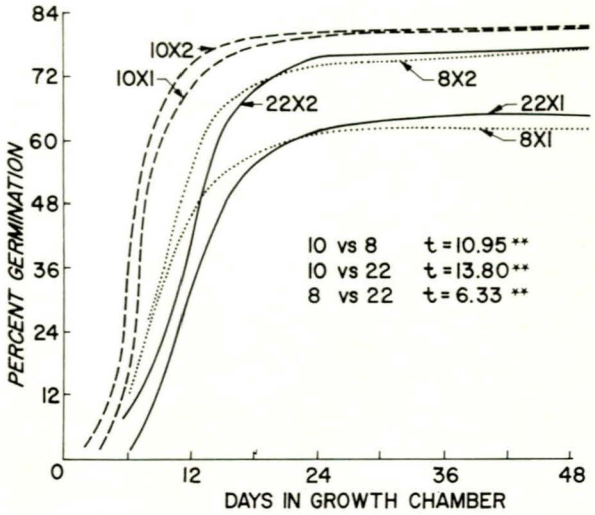


FIGURE 2. — Maternal influence of clones 8, 10, and 22 on germinative energy. ** indicates significant at the 1-percent level.

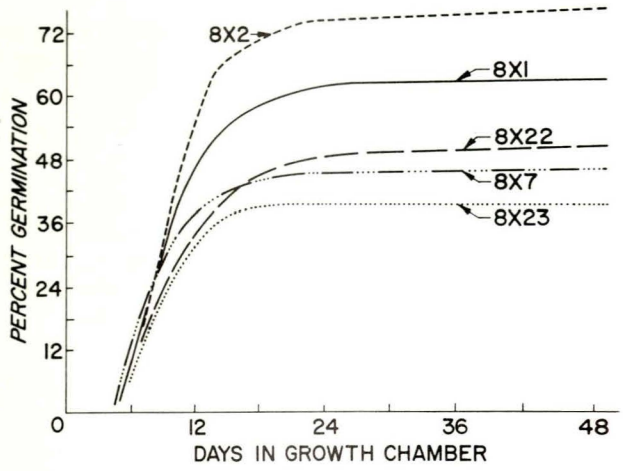


FIGURE 3. — Paternal influence on total germination.

these tests, may have little significance so far as natural regeneration is concerned. In a wild stand, where we cannot moderate the weather, we never know whether nature will favor an early or a later germinating lot in a given year. Furthermore, if nature had been partial to either when selecting survivors over the past few thousand years, we would expect less variation among trees growing in a relatively limited area.

For artificial regeneration, however, high germinative energy may be a decided advantage. In the nursery, prompt and even germination can get most of the seedlings off to an even start and produce uniform stock that simplifies the nurseryman's problems. In direct seeding also, high germinative energy could permit quick response of the seeds to favorable growing conditions. This could be particularly helpful on south slopes where favorable conditions may be of very limited duration. Furthermore, quick germination could reduce the time during which seeds are subject to bird and rodent damage.

# VARIATION AND INHERITANCE OF SOME PHYSIOLOGICAL AND MORPHOLOGICAL TRAITS IN DOUGLAS-FIR

by Oscar Sziklai[1]

Forest genetics is the study of variation and heritability in forest trees. It is concerned with similarities and differences of various traits between related trees and their transmittance to the next generation.

Variation itself is a product of differences between individuals and the effects of environmental modifications, genetic recombinations and mutations (Stebbins 1957). An understanding of the role of the above factors is essential in any genetic studies. The variation pattern from tree to tree and from stand to stand as well as the population composition throughout the range of the species must be known before any improvement work can be planned on a logical basis. The calculation of genetic gain requires an estimate of heritability which will express the probability that certain characteristics will appear in future generations. Only limited information is available at present on the inheritance of various traits of forest tree species.

## Materials and Methods

Four Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco var. *menziesii*) trees were selected on the U.B.C. Campus and designated A, B, E, and 11. Trees A, B, and E originated from natural regeneration and represent the local population of Douglas-

[1]*Associate Professor, University of British Columbia, Faculty of Forestry, Vancouver.* *Financial support was provided by the National Research Council of Ottawa (67-1375) and the Committee on Research, University of British Columbia (68-0316).*

fir, while tree 11 was selected from a plantation established in 1934, from an unknown coastal provenance.[2]

Phenological observations on the flushing date of the vegetative bud were carried out for 4 years, commencing in 1959.

Isolation of the megasporangiate strobili was carried out 2 to 3 weeks prior to pollination. For isolation, viscose casings were used as recommended by Duffield (1950). Pollen was extracted by a modification of the method described by Orr-Ewing (1954) and was stored at 0° C. until required.

Three different pollination methods were used: dry, wet, and dry-wet as described by Allen and Sziklai (1962), in all, 16 combinations. Isolation bags were replaced by fiber-glass screens 6 to 8 weeks after pollination to protect the developing conelets from insect damage.

After collecting the cones during the first half of September, the seeds were extracted, counted, and separated by weight into two classes—filled seed and empty seed. Seven milligrams were used as the lower limit for filled seed. Germination tests of filled seed, using the method described by Allen and Bientjes (1954), were run for 40 days at a constant temperature of 25° C. Light was not applied, except for a short period every day when the germinants were removed.

Later, 6 average germinants were transplanted into a plastic container (100 x 100 x 135 mm) filled with vermiculite to a depth of 130 mm. Irrigation with Hoagland's solution was provided daily. Long-day environmental condition was established in a Percival PGC-78 growth chamber with 15 hours of illumination and with a light intensity of 3500 foot-candles. The temperature during the illumination period was 25° C., while 15° C. was maintained during the dark period. Although the relative humidity was not controlled, it correlated closely with temperature changes; 50-60 percent relative humidity occurred during the illumination period and 80-100 percent during the dark period.

Each cross was represented by 6 seedlings grown in one container in the chamber. The containers were placed randomly on the shelves of the growth chamber and were rearranged randomly every 10-14 days to further reduce biases that might have been caused by variations in the distance from lights, observation windows, or circulation fans.

Although the repeated moving of the containers reduced environmental variation and provided some randomization, the experimental design was not randomized and the results and analyses must be considered with some reservation. However, it is felt that they are of sufficient interest to warrant publication. Furthermore, other tests (which space does not permit publishing at this time) have verified the results in most respects.

[2]*Personal communications from F. M. Knapp, 1963.*

The progeny test in the growth chamber ran from February 25, 1963, to July 6, 1963, a period of 132 days. The seedlings were then examined for: length of roots to 1.0 mm, length of hypocotyl to 1.0 mm, length of epicotyl to 1.0 mm, length of branches to 1.0 mm, number of branches, number of cotyledons, diameter of root collar to 1.0 mm, green weight of shoots to 0.1 g, dry weight of roots to 0.001 g, dry weight of shoots to 0.001 g, light transmittance of chlorophyll.

The transmittance of chlorophyll was measured, using the method described by Madison and Anderson (1963). One gram sample of needles was extracted in a 20 ml. methanol. The needles were collected from the middle portion of the shoot and were cut into approximately one-mm lengths to provide better penetration of methanol. A Beckman colorimeter was used, with a filter to pass wave lengths of about 440 millimicrons. An extract of needles of open-pollinated progeny from tree E was used as the standard. The readings were obtained in percent and later on were transformed to arcsine values for analysis.

## Methods of Statistical Analysis

The individual seedling records were used in the calculation, which is based on the following mathematical model:

$$x_{ijk} = m + a_{s_i} + b_{p_j} + c_{sp_{ij}} + e_{ijk}$$

where: $x_{ijk}$ = the observation of the k-th offspring of a cross, in which the seed (female) parent belonged to the i-th tree, and the pollen (male) parent belonged to the j-th tree.

$i$ = the number of trees used as seed parent,

$j$ = the number of trees used as pollen parent,

$k$ = the number of seedlings from each cross,

$m$ = the sample mean,

$a_{s_i}$ = the effect of the i-th seed parent,

$b_{p_j}$ = the effect of the j-th pollen parent,

$c_{sp_{ij}}$ = the interaction of the i-th seed and the j-th pollen parent,

$e_{ijk}$ = the error term

The expected mean squares were derived from analysis of variance (table 1) and the component of variance was calculated, using the following forms for each analysis.

When the mean square of the seed-pollen (S x P) interaction was less than the mean square of the residual variance, its component of variance was considered as non-existent. To obtain more accurate estimates of the residual variance, the two sums of squares and degrees of freedom were pooled and

Table 1.--Analysis of variance and component of variance using individual seedlings from a polyallel cross of Douglas-fir

| Source of variation | Degree of freedom 1/ | Components of variance 1/ |
|---|---|---|
| Seed (S) | i-1 | $MS_s = S_e^2 + kS_{sp}^2 + kj\ S_s^2$ |
| Pollen (P) | j-1 | $MS_p = S_e^2 + kS_{sp}^2 + ki\ S_p^2$ |
| S x P | (i-1)(j-1)-1' | $MS_{sp} = S_e^2 + kS_{sp}^2$ |
| Residual | ijk-ij-(k-1)1' | $MS_e = S_e^2$ |

1/ Explanation of symbols is as follows:

$S_s^2$ = the genetic component of the seed parents

$S_p^2$ = the genetic component of the pollen parents

$S_{sp}^2$ = the genetic component from interaction of seed and pollen parents

$S_e^2$ = the residual variance, which cannot be allocated to the other three terms

1' = the number of selfings from polyallel cross

a new residual mean square was calculated. The heritability coefficient was calculated for the seed and pollen parents separately by dividing the genetic-variance components of each parent with the phenotypic variance:

$$\text{for seed parents: } h_s^2 = \frac{S_s^2}{S_s^2 + \frac{S_{sp}^2}{i} + \frac{S_e^2}{ik}}$$

$$\text{for pollen parents: } h_p^2 = \frac{S_p^2}{S_p^2 + \frac{S_{sp}^2}{j} + \frac{S_e^2}{jk}}$$

## Phenological Differences

Vegetative bud flushing was consistent, and significant tree-to-tree differences were demonstrated. Tree B was consistently earliest and tree 11 latest in flushing during 4 years (table 2). The difference was 16 days on the average. Griffith noted a maximum difference of 5 weeks between early- and late-flushing trees among the 154 trees observed at the University Research Forest, near Haney, B.C.[3] Early-flushing tree B appears to be subject

Table 2.--The average date of vegetative bud-flushing in 1959, 1960, 1961, and 1963

| Year | Tree number | | | | Average |
|---|---|---|---|---|---|
| | A | B | E | 11 | |
| | Number of days since January 1 | | | | |
| 1959 | 150 | 143 | 148 | 157 | 149.5 |
| 1960 | 140 | 132 | 136 | 154 | 140.5 |
| 1961 | 148 | 141 | 144 | 155 | 147.0 |
| 1963 | 146 | 139 | 139 | 153 | 144.3 |
| Average | 146.0 | 138.7 | 141.7 | 154.7 | |

to greater variation (SD = ±4.79 days) than the late-flushing tree 11 (SD = ±1.73 days).

Although flowering characteristics were not systematically observed, notes on pollination reveal that the times of flowering and flushing were not necessarily correlated (r = .24):

| *Tree number* | *Pollination: Number of days since January 1* |
|---|---|
| A | 108 |
| B | 99 |
| E | 103 |
| 11 | 102 |

Ripening of pollen flowers started a few days before the first ovulate flowers reached the fully receptive stage. This slight protandry was also

[3] *Griffith, B. G. 1963. Phenology, growth, and flower and cone production of open-grown Douglas-fir trees. In manuscript, University of British Columbia.*

observed by Orr-Ewing (1956) and Stoate *et al.* (1961). Simultaneous maturation of the male and female flowers (synacme) dominated the large part of the flowering period, and would allow self-pollination.

## Results of Controlled Pollination

Of the pollinated ovulate conelets, 201 out of 290 developed into cones in 1962, and 193 out of 413, in 1964. In both years, the loss was largest, 58 and 52 percent respectively, on the earliest (B) and latest (A) flowering trees. The loss on tree 11 (average of 17 percent) was the least in both years.

The different pollination methods used in cross-pollination (table 3) gave cone mortalities of 49 percent for the dry method, 34 percent for the dry-wet method, and only 19 percent for the wet method. When selfing, only the wet pollination method was used and the loss of conelets was 24 percent at the end of the growing season.

Specific gravity of the pollen grains in Douglas-fir is larger than one, and the pollen could sink close to the integument of the ovule when wet pollination is applied and water is accumulated between the ovuliferous scales and the bracts. That water also does not interfere with the stigmatic inner surface of the integument during the pollination process was found by Allen and Sziklai (1962). Silen and Krueger (1962) indicated that rain during the pollination period could not cause a major reduction in Douglas-fir seed set. The number of filled seed per cone on tree B in 1962 was 19.00 when dry, 19.38 when wet, and 26.71 when dry-wet pollination methods were used.

To further investigate seed production, artificial pollination was separated into cross- and self-pollination groups, and wind-pollination was also included (table 4).

Since the development of seed does not depend on pollination, the total number of seeds per cone should not differ greatly on the same tree as a result of different pollination methods. Although the different average values among the total number of seeds did not differ significantly, wind-pollination resulted in only 30.87 seeds per cone, compared to 37.09 for self- and 42.42 for cross-pollination. The low number of seeds per cone in wind-pollination may be attributed to the damage caused by *Contarinia oregonensis* Foote. The seeds become fused with the scale and are not released from the cone (Hedlin 1958).

The number of filled seeds per cone from cross-pollination (13.81) is significantly different from

Table 3.--Number of ovulate conelets isolated and cones collected, using the various pollination methods in 1962

| Method of pollination | Tree number | | | | | | | | | | Conelets lost (percent) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | A | | B | | E | | 11 | | Totals | | |
| | Poll. | Coll. | Poll. | Coll. | Poll. | Coll. | Poll. | Coll. | Poll. | Coll. | |
| Dry | 15 | 2 | 17 | 7 | 12 | 6 | 20 | 18 | 65 | 33 | 49 |
| Wet | 22 | 20 | 19 | 16 | 11 | 9 | 21 | 14 | 73 | 59 | 19 |
| Dry-wet | 18 | 7 | 15 | 7 | 19 | 17 | 15 | 13 | 67 | 44 | 34 |
| Self-wet | 23 | 18 | 18 | 10 | 17 | 15 | 27 | 22 | 85 | 65 | 24 |
| Total | 79 | 47 | 69 | 40 | 59 | 47 | 83 | 67 | 290 | 201 | 31 |

Table 4.--Average total and filled seeds per cone from cross-, self-, and wind-pollination in 1962

| Tree no. | Cross | | Self | | Wind | |
|---|---|---|---|---|---|---|
| | Total | Filled | Total | Filled | Total | Filled |
| A | 38.8 | 9.6 | 38.8 | .4 | 37.1 | 2.0 |
| B | 42.2 | 21.0 | 39.5 | .0 | 23.0 | 1.8 |
| E | 53.4 | 2.7 | 45.5 | .9 | 32.7 | 4.1 |
| 11 | 35.3 | 21.9 | 24.5 | 6.3 | 30.6 | 4.3 |
| Average | 42.42 | 13.81 | 37.09 | 1.91 | 30.87 | 3.05 |

self- (1.91) and from wind-pollination (3.05) (table 4). Compared with wind-pollination, cross-pollination increased the number of filled seeds 4.8 times on tree A, 5.1 times on tree 11, and 11.4 times on tree B. However, the wind-pollination method produced more filled seeds than did cross-pollination on tree E, probably because the controlled pollination took place too late in 1962.

Application of artificial pollination in seed production areas, and especially in seed orchards, has obvious potential value in tree improvement programs.

Self-pollination gave the lowest average number (1.91) of filled seed per cone (range from 0 to 6.27). Orr-Ewing (1956) indicated a range between 0.37 and 13.82, and pointed out that self-pollination might result in the same number of filled seeds as in cross-pollination. On the other hand, certain self combinations might produce only empty seeds, as on tree B. Self-pollinations were attempted in previous years on tree B, but filled seeds were never obtained. It is logical to assume that deleterious mutant recessive genes have accumulated in *Pseudotsuga*. Since this is an outcrossing species, the appearance of these genes is hidden by the corresponding dominant alleles. In self-pollination (inbreeding), these genes could arrive at a homozygous condition, and their effect could be clearly expressed in the seeds or progeny. It is also possible to assume that some of these recessive genes are lethal and are able to block fertilization or destroy the embryo shortly after fertilization takes place.

## Germination Tests

In the germination tests on seeds from controlled crosses all of the above-mentioned variations in environmental factors were eliminated or reduced. This helped to bring to light the heritability variances.

The seed germination percent varied by mother tree as follows: E, 91.3; A, 76.2; D, 74.1; and 11, 42.0. When tree E pollen was applied, a high germination value was observed in every case. On tree E, no change in germination percent was evident in any cross when a different pollen source was used, indicating a high combining ability for this tree.

## Progeny Test in the Growth Chambers

Data available from the polyallel crosses permitted an assessment of the combining ability of the four Douglas-fir trees, both as seed and as pollen parents. Tree 11 appears to be the best seed parent, followed by tree E, B, and A. As a pollen partner, tree B proved to be the best, followed by tree 11, A, and E. Tree 11 as a pollen parent was not far behind tree B. In a few important characteristics, such as length of epicotyl, diameter of root collar, and green and dry weight of shoot, the crosses with pollen from tree 11 surpassed the crosses with pollen from tree B.

The combining ability of the parents provides evidence that intraspecific crosses can yield significantly different positive results in 132-day-old progenies of Douglas-fir. The polyallel cross in connection with a short testing method, using growth chambers, seems very useful, but will have to be modified to best fulfill the requirements of forest tree improvement.

The correlation coefficients between total height of 4-year-old and the epicotyl lengths of 132-day-old progenies were calculated, using the four available 1958 crosses—B x A, B x E, E x A, and E x B. A significant correlation was found with a value of 0.895 ($r_{05}=0.879$ and $r_{01}=0.959$, $df=3$). The first year's total height growth also showed significant or highly significant correlation between total heights after 1 year and after 4 years of the same crosses. This may mean that the effect of the seed is not well pronounced in these crosses, or diminishes at a very early stage of development. On the other hand, the correlation between shoot (epicotyl) length at 132 days and the total height after 4 years is a useful one, and promises a certain time reduction in progeny testing in forest tree breeding, where time is one of the greatest obstacles.

It was possible to calculate narrow-sense heritability values only for length of epicotyl (seed parent; 0.36), length of branches (seed parent; 0.72, pollen parent; 0.58), number of cotyledons (pollen parent; 0.42), green weight of shoots (pollen parent; 0.22) and for light transmittance of chlorophyll (seed parent; 0.98, pollen parent; 0.97).

The moderate inheritance in epicotyl length attributed to seed parents (0.36) is important in forestry practice. By using such seedlings, rapid juvenile growth may overcome brush competition earlier and the new forest stand could be established more quickly and surely. At the end of the 132-day experimental period, for instance, seedlings had grown to average heights, by crosses, of 224.3 mm for 11 x A, 129.3 mm for B x A, and 126.0 mm for E x A. In all these crosses, the pollen parent was the same; only the seed parents were different. By selecting seedlings from 11 x A crosses, the increase could be between 73 and 78 percent in comparison to seedlings from B x A or E x A crosses respectively. Such trees could be propagated in seed orchards to give a quick increase in seedling height. This might be worthwhile even if we are taking a calculated risk in not knowing the correlation between seedling and mature tree height.

The length of branch was under strong genetic control, and the seed parent effect was larger than that of the pollen parent.

Generally, except for the light transmittance of chlorophyll, the heritability estimates are moderate, which confirms the assumption of Toda *et al.* (1959) that in many characteristics the additive

genetic variance is small compared to other variances. The moderate heritability values also indicate that selection based on phenotypic values may not be a reliable indication of the success of such a breeding program. The writer wholeheartedly agrees with the statement of Wright *et al.* (1958): "Most significant improvement will come from programs in which every parent is carefully selected and carefully progeny-tested and in which controlled pollination plays a major part."

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# THE PRODUCTION OF HOMOZYGOUS TREE MATERIAL


by Reinhard F. Stettler and George E. Howe[1]


Homozygous trees will never be the desired ultimate step in a forest tree improvement program. However, they will serve many purposes in forest genetics research: (1) in the detection of genetic markers; (2) in the isolation of traits under simple genetic control for the study of growth and differentiation phenomena; (3) as a tool as well as reference material in the study of breeding mechanisms in natural populations; (4) as reference material in a study of the efficiency of different selection schemes; and (5) in the study of sex control mechanisms in dioecious species. Furthermore, homozygous trees could serve as an intermediate step for the production of heterotic intra- and interspecific hybrids.

The inbreeding approach to the production of homozygotes is inefficient in trees. An alternate approach is the induction of haploid parthenogensis followed by colchicine duplication of the haploid chromosome set. In fact, recent evidence by Muntzing (1963) indicates that this approach may be the only way to produce true homozygosis in an entire genome. He found segregation at a conspicuous locus in rye still after 30 generations of selfing and suggested that heterozygosity may have been preserved in many additional, more concealed traits.

The first successful attempt to apply haploid induction to forest-tree material was reported by Kopecky (1960), who recovered 11 haploid plants after pollinating *Populus alba* L. with pollen from *P. tremula* L. and *P. nigra* L. Meanwhile, several researchers have indicated they are engaged in studies of this kind, and we can expect additional information on the subject.[2]

## Choice of Organism

For a number of reasons, *Populus trichocarpa* Torr. & Gray, the native black cottonwood, seemed best suited as experimental material for our studies. The genus *Populus*, a member of the *Salicaceae*, has a uniform chromosome number of $2n = 38$. Even though this number indicates that polyploidy may have played a role in the past evolution of the genus, chromosome behavior suggests that the present-day material can be considered diploid (Dillewijn 1940). More gain in efficiency


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can be expected from applying the haploid method to a diploid than to a polyploid organism.

All poplars, with the exception of *P. lasiocarpa,* Oliv. are dioecious wind pollinators. However, occasional hermaphroditic individuals have been reported in several poplar species and interspecific hybrids (reviewed by Lester 1963). A higher degree of heterozygosity is to be expected in natural populations of dioecious than of monoecious organisms. Artificially produced homozygotes in a dioecious organism would allow a wider range of comparison regarding the relative effects of homo- vs. heterozygosity. Furthermore, the production of homozygous stock in poplars may give insight into the genetic control of sex expression in this genus. It is realized that this advantage may be offset by a correlated disadvantage of imbalanced sex distribution among artificially produced homozygotes.

The mechanics of poplar breeding are simple. Flowering branches can be removed from the trees and kept in water culture in the greenhouse from pollination to seed harvest. This makes it possible to keep large numbers of potential embryos under experimental conditions in a relatively small area: approximately a half-million ovules can be grown in an area of 150 square feet. The period from pollination to mature seed is short enough to keep environmental variability at a minimum. Once the seed is harvested it germinates readily (no dormancy), thus allowing early scoring of progenies. Dormant branches can be induced to flower as early as January; alternatively they can be kept dormant in cold storage until May. Both methods help to extend the period during which haploid induction experiments can be conducted.

Many poplars, including *P. trichocarpa,* are ideally suited for cloning. Once haploids have been recovered, they can be propagated vegetatively, thus increasing the probability that some of them will be diploidized successfully. Black cottonwood occurs both in small, isolated, as well as in large, continuous populations covering a wide range of distribution. Much genetic diversity can be expected that may have a bearing on its response to haploid induction.

Lastly, *Populus* is one of the few tree genera in which haploids have been reported (Kopecky 1960; Tralau 1957). If we are to gain insight into those aspects of reproductive physiology that are associated with haploid parthenogenesis, it seems advantageous to study an organism in which the phenomenon can be expected to occur.

## Experimental Considerations

While our long-term program aims at the mass production of haploids for subsequent establishment of homozygous stock, our immediate objective is to develop a repeatable technique of stimulating haploid parthenogenesis in female tissue of black cottonwood. This technique may ultimately consist of using pollen of a particular poplar species on black cottonwood; it may possibly involve the use of irradiated pollen, chemical agents, environmental shocks, or any combination thereof. Regardless of the specific nature of the ultimate technique, it is safe to predict that it will be more successful in certain genotypes than in others (Chase 1952).

Since the female tissue offers more diversity for experimental manipulation than the male, a screening for responsive genotypes seems more justified in the female than in the male sex. The first step in the experimental procedure should therefore be a screening for responsive females with the aid of the most generally successful induction method. Once responsive females have been singled out, a variety of induction methods could be tested on them.

Since the majority of experimentally induced haploids in angiosperms, including those in *Populus alba,* have been recovered after hybridization (reviewed in Kimber and Riley 1963), it was decided to screen for responsive females in *P. trichocarpa* after they had been exposed to pollen of a number of other poplar species (*P. nigra, P. deltoides* Bartr., *P. canescens* (Ait.) Sm., *P. grandidentata* Michx., and *P. tremuloides* Michx.).

For lack of information it is assumed that the probability of recovering haploid seedlings following hybridization in non-selected female genotypes of *P. trichocarpa* is of the same order of magnitude as in other angiosperms, i.e. approximately 0.1 percent of the number of ovules involved (Kimber and Riley 1963). In the absence of known seedling markers, it is further assumed that 50 percent of the viable haploids are not recognized. On the assumption that the frequency of ovules giving rise to observable haploid seedlings follows a Poisson distribution, it can be calculated that it takes 6,000 ovules to detect one or more haploids with 0.95 probability (Burington and May 1953). This figure serves as the basis for calculating the required number of branches per genotype to be tested with each pollen species.

Last, it seems reasonable to suspect that the past breeding history of a population will be reflected in the response by its members to haploid induction. *A priori,* one would expect a higher probability of haploid survival in small, isolated populations than in large, continuous populations. A comparison of females derived from these two extreme conditions would therefore be desirable.

## Pilot Studies

Pilot studies were required to familiarize ourselves with culture and breeding techniques and to solve some physiological problems associated with our induction method. One phenomenon, in particular, posed a serious problem, namely, the

premature abscission of female inflorescences after treatment with either no pollen or foreign species pollen, especially in the case of wide crosses.

In 1964 we carried out experiments to study the effects of IAA treatment in preventing premature inflorescence abscission. Branches from three different female trees were treated one day after pollination with different concentrations of IAA in two different media (water, lanolin). While the auxin treatment successfully delayed inflorescence abscission in one genotype, it did not allow the inflorescences to reach mature-seed stage. No effects were observed in the other two genotypes.

In 1965 we tried another approach by making use of observations reported by Brewbaker (1962), Stairs and Mergen (1964), and a number of other researchers, that genetically inactive pollen may still have sufficient physiological activity to perform some stimulatory function. Their observations had been based on the study of pollen having received high doses of acute ionizing radiation.

For this purpose, we exposed *P. trichocarpa* pollen to a $Co^{60}$ source for a dose of 100,000 r. This pollen was then tested as *agens stimulans* (a) singly, and in combination with pollen of (b) *P. trichocarpa,* (c) *P. deltoides,* and (d) *P. canescens.* Parameters studied included the percentage of inflorescences reaching mature-seed stage; the number and size of ovules as well as the number of embryos per pistil at different stages during development; nuclear size and chromosome number in embryos and seedlings. The results have been very encouraging and can be summarized as follows:

1. Irradiated pollen, singly or in combination, increased the percentage of inflorescence reaching maturity. In fact, 48 percent of the inflorescences treated with only irradiated pollen were retained to the mature stage.

2. Irradiated pollen stimulated the growth of pistils and of many of the ovules contained therein.

3. In mixture with normal *P. trichocarpa* pollen, the irradiated pollen was sufficiently competitive to significantly decrease the number of diploid embryos per pistil. This suggests that the tubes of the irradiated pollen successfully grew to many of the receptive ovules and prevented them from being fertilized by normal pollen.

4. No mature haploid embryos were found among the ovules treated with irradiated pollen only. This supports the findings of Ehrensberger (1948) that the survival of haploids in angiosperms depends on the presence of a functional endosperm.

At the University of Washington, studies are in progress to further investigate the behavior of irradiated poplar pollen, singly and in mixture with normal or foreign species pollen, to better understand its physiology. This will help us to evaluate its usefulness not only as a tool in our haploid induction program but also as a tool in intra- and interspecific hybridization.

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# TECHNIQUE AND INTERPRETATION IN TREE SEED RADIOGRAPHY

by Howard B. Kriebel[1]

The study of internal seed structure by radiography requires techniques which will give good definition. To establish the best procedures, we conducted a series of experiments in which we manipulated the principal controllable variables affecting the quality of X-radiographs: namely, focus-to-film distance, film speed (grain), exposure time, kilovoltage, and milliamperage. The results presented here will eliminate the need for repetition by others interested in moving directly to research applications of X-radiography. A few examples will illustrate the interpretation of detail and perhaps suggest how radiographs can contribute to an understanding of the biology of seed formation.

The physics of X-rays and fundamental principles of radiography are clearly described in illustrated publications by Eastman Kodak (1957, 1959, 1960) and General Electric (1957, 1963). Pictures obtained by this diagnostic method are, strictly speaking, radiographs and not "X-ray photographs", because light is not the type of radiant energy involved except in certain special methods.

This exposure to radiation is for diagnostic purposes only. It will usually be applied at a very low dosage level, with so-called "soft" X-rays, rather than the "hard" X-rays used for inducing changes in somatic or reproductive cells. The X-ray machine used should be designed for radiography and for operation at low kilovoltages.

Studies of radiation sensitivity of tree seeds, especially of pines, have given some indication of minimum dosage levels of X-rays which will affect germinability or induce somatic changes in the plant. These studies have included investigation of certain seed properties and conditions influencing radiosensitivity (Baldwin 1936; Simak and Gustafsson 1953; Gustafsson and Simak 1958; Suszka *et al.* 1960; Simak *et al.* 1961; Ohba and Simak 1961; Ohba 1961; Snyder *et al.* 1961; Yim 1963). Results obtained with various conditions of moisture content, seed ripeness, post-treatment storage time, and other factors indicate that in pines the 100 to 600r range is the lowest at which any depressing effect on germination rate or time can be observed. On the basis of present knowledge, the 1 to 3r dosage required for most coniferous seed radiography appears to present a very low risk of tissue damage or induced mutations.

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Various aspects of the application of X-ray analysis to tree seed testing have been discussed in the literature. The technique is useful for evaluating seed maturity and quality, insect infestation, and mechanical damage. In certain species the technique has been refined for viability testing by the use of organic and inorganic seed-impregnating contrast agents. These applications have been discussed by various workers, including Muller-Olsen and Simak 1954; Simak 1955, 1957; Muller-Olsen *et al.* 1956; Gustafsson and Simak 1956; Rohmeder 1957; Hagner and Simak 1958; Nekrasov and Smirnova 1961; Swaminathan and Kamra 1961; Simak and Kamra 1963; Kamra 1963a, 1963b, 1964a, 1964b; Hansen and Muelder 1963. Results of a study by Klaehn and Wheeler (1961) indicated that examination of remnants of the embryo and the collapsed female gametophyte in so-called "empty" seed may provide information on the degree of incompatibility in species crosses and selfings.

## Equipment

X-ray units designed specifically for seed radiography, in particular for checking stored grain for insect infestation, were formerly available, but their production has been discontinued. The two principal types of machines now available for radiography are the medical and industrial units. Medical types are mainly designed for very rapid exposures with high current and are generally not as suitable for tree seed radiography as are some of the industrial units.

The type used in these studies was a General Electric LC-90 Industrial X-Ray Unit with a CSI-25 tube. This unit has a range of 0-40 kvp, 1-5 ma, has a nominal focal spot size of 2 mm. and 0.030 beryllium filtration. We have enclosed it in a lead housing large enough to permit a maximum focus-film distance of 28 inches.

## Films

Table 1 lists the characteristics of some X-ray films suitable for tree seed radiography. It is not intended to be a complete list of all brands or types available. Data are from publications of Eastman Kodak Company and Picker X-Ray Corporation. Medium and coarser grain fast films are also available, but these types are less suitable for seed radiography.

## Experimental Procedure and Results

Experiments with radiographic technique included the following variables:

Table 1.--Grain, contrast, and speed of some x-ray films suitable for seed radiography

| GROUP | GRAIN | CONTRAST | RELATIVE SPEED 1/ | BRAND TYPE |
|---|---|---|---|---|
| I | ULTRAFINE | VERY HIGH | VERY SLOW (10) | KODAK R (SINGLE-COATED) |
| II | ULTRAFINE | HIGH | SLOW (20) | KODAK R<br>ANSCO SUPERAY HD |
| III | VERY FINE | HIGH | SLOW (40) | KODAK M<br>ANSCO SUPERAY B<br>DUPONT 510<br>ILFORD F |
| IV | FINE | FAIRLY HIGH | MEDIUM (150) | KODAK AA<br>ANSCO SUPERAY A<br>DUPONT 506<br>ILFORD B AND C |

1/ NUMBERS ARE FOR KODAK FILMS, AS RATED BY EASTMAN KODAK FOR ALUMINUM AND OTHER LIGHT ALLOYS, NO SCREENS, 80 KV.

Type of film (5 types, groups II, III, IV)
Kilovoltage (10, 12½, 15, 17½, 20, 25, 30)
Milliamperage (1, 3, 5)
Focus-film distance (5½", 9", 12½", 19", 28")
Time (15 to 360 seconds)

It was not necessary to include every possible combination of factors. It soon became evident, for instance, that the higher range of kilovoltage could only be used at the longest distance, with an exposure of very short duration. The number of practical treatment combinations for any particular type of film is therefore rather limited.

Under constant conditions of kilovoltage, milliamperage, and target-film distance, image intensity varies in direct linear proportion to exposure time. This relationship can be measured by recording dosage in roentgens per unit of time. Figures 1 and 2 illustrate results obtained at 3 ma using distances of 19.5 and 28 inches, and kilovolt peaks of 15, 17.5, and 20. Repetitive readings were taken with a Victoreen Model 570 Condenser R-Meter with an interchangeable ionization chamber.

Radiation intensity varies inversely with the square of the focus-film distance, if other factors are constant. Actual dosage curves obtained with

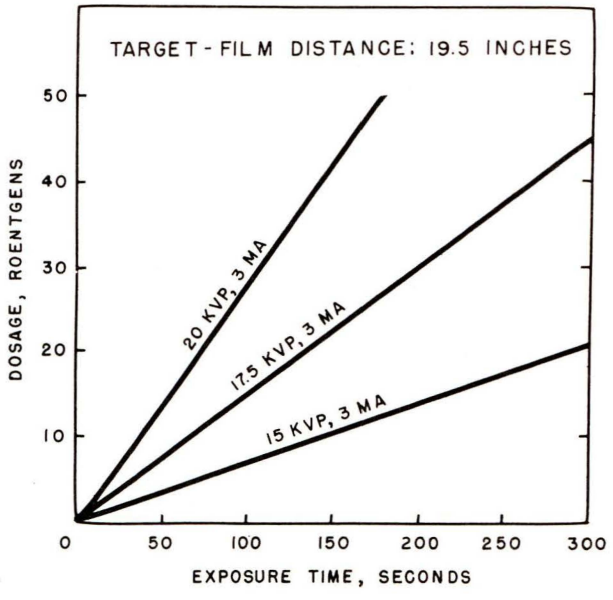



FIGURE 1. — The relationship of radiation intensity at the film plane to exposure time and kvp, at 3 ma and 19.5 inches target-film distance (GE CSI-25 tube, 2 mm. focal spot, 0.030 beryllium filtration).

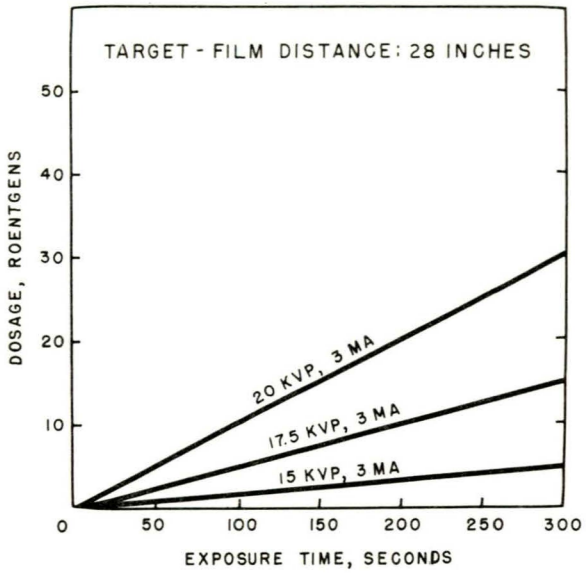



FIGURE 2. — The relationship of radiation intensity at the film plane to exposure time and kvp, at 3 ma and 28 inches target-film distance (GE CSI-25 tube, 2 mm. focal spot, 0.030 beryllium filtration).

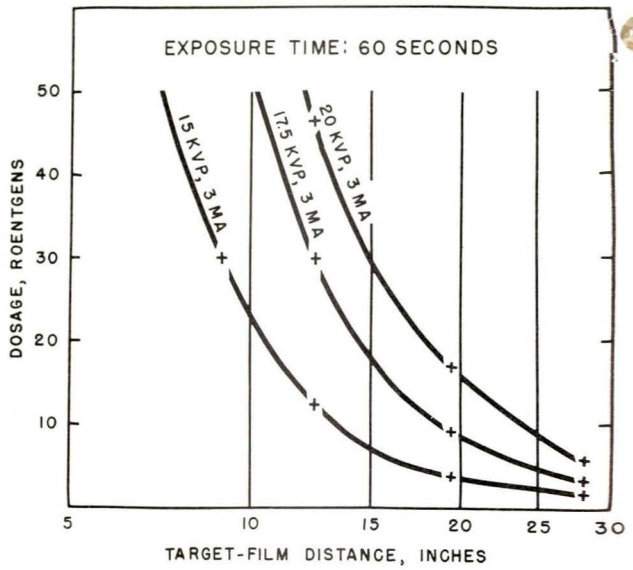



FIGURE 3. — The relationship of radiation intensity at the film plane to target film distance and kvp, at 3 ma with a 60-second exposure (GE CSI-25 tube, 2 mm. focal spot, 0.030 beryllium filtration).

the meter are shown in figures 3 and 4, for combinations of 3 and 5 ma with 15, 17.5 and 20 kvp for a 60-second exposure.

Theoretically, the milliamperage required for a given exposure is inversely proportional to the exposure time. This means that the product of milliamperage and time always remains constant for a given radiographic result, if no other factors are changed (Eastman Kodak Co. 1960). Under this rule an increase in milliamperage should increase exposure, other factors being equal. The rule holds true at the high kilovoltages normally used in industrial and medical radiography. However, when the kilovoltage is less than 22 or 23, there is a reversal, at least with the General Electric LC-90, CSI-25 unit. A greater intensity of radiation is obtained at 15 or 20 kvp with 3 ma than with 5 ma. This relationship can be seen in figures 3 and 4. Dosages are higher in each curve at any distance at 3 ma than at 5 ma, although as the kilovoltage increases, the difference decreases.

There is noticeably less contrast in images made at 20 kvp than in those made at 15 kvp. High contrast seems to give the best definition for seed radiography, and for this reason and in order to minimize dosage, 15 kvp appears to be about the optimum level, at around 3 ma. Also for reasons of definition and dosage level, we consider our maximum obtainable distance of 28 inches to be most satisfactory for coniferous seed.

We have, therefore, selected 15 kvp, 3 ma, and 28 inches as constants for most of our pine seed radiography. The level of the other variable, time,

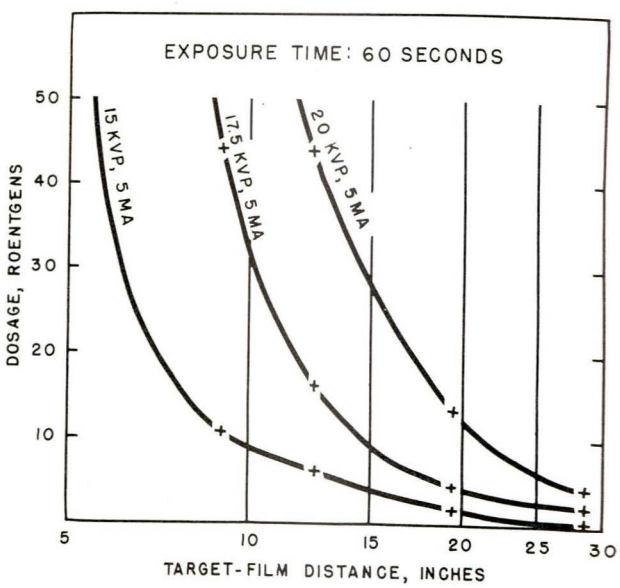



FIGURE 4. — The relationship of radiation intensity at the film plane to target-film distance and kvp, at 5 ma with a 60-second exposure (GE CSI-25 tube, 2 mm. focal spot, 0.030 beryllium filtration).

is determined by the type of film used. Optimum exposure times for films tested under these conditions are presented in figure 5. These values are based on inspection of several hundred radiographs by 2 observers at 2½ magnifications.

Very little gain in definition of detail is obtained by using a "very fine" or "ultrafine" grain film

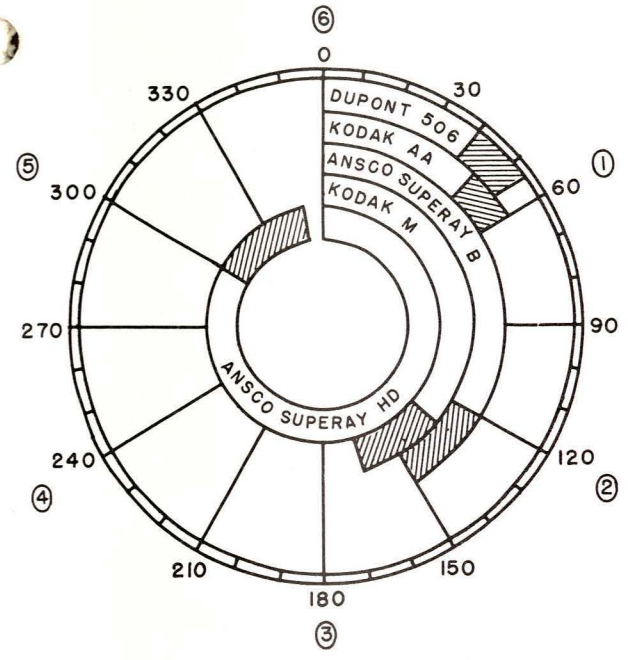



FIGURE 5. — Optimum exposure times for high definition in white pine seed radiographs, with dosage levels, using various films at 15 kvp, 3 ma and 28 inches focus-film distance (GE CSI-25 tube, 2 mm. focal spot, 0.030 beryllium filtration).

in place of a "fine" grain film (table 1). Sharpness of image detail is less dependent on film grain than it is upon the distance between subject and film plane. If the seeds are placed directly on top of the shielded film, nothing can be done to reduce this distance. With large seeds, the undesirable effect of greater subject-to-film distance is offset by the large size of the seed image. Film grain is a relatively unimportant consideration under these conditions. Radiographs of very small seeds, on the other hand, may require inspection at 5 to 10 or more magnifications. In this case there may be an advantage in the use of the finer grain films, with longer exposure time and higher radiation dosage. Of course, if the seed is not to be carried through germination, dosage level is of no importance.

## Examples of Interpretation of Seed Radiographs

Figure 6A is typical of detail obtainable in radiographs of white pine seeds under conditions just described. This is seed from an intraspecific cross. All filled seeds are normal and fully developed.

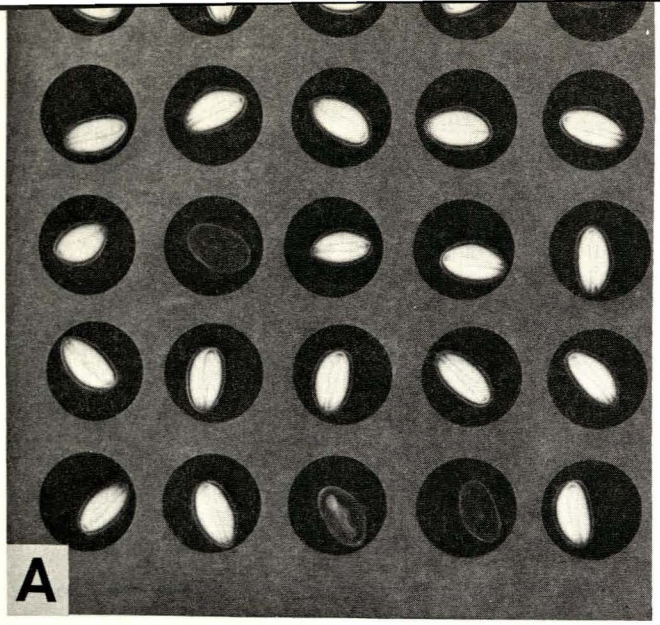
In our crossing program, sound seed from selfing of *Pinus strobus* look just the same as the seed in figure 6A. The entire seed lot, of course, has a much higher proportion of empty seed than would be obtained from outcrossing, but defective or poorly developed seeds have seldom been found after selfing white pine. The filled seeds have the same high germination capacity as cross-pollinated seeds, they germinate promptly, and exhibit slight inbreeding depression in the seedling stage. Apparently the incompatibility reaction is lethal at an early stage. These results could be quite different from those obtained in other breeding programs and could be expected to vary with the species or species group and the degree of inbreeding already present in the selfed tree.

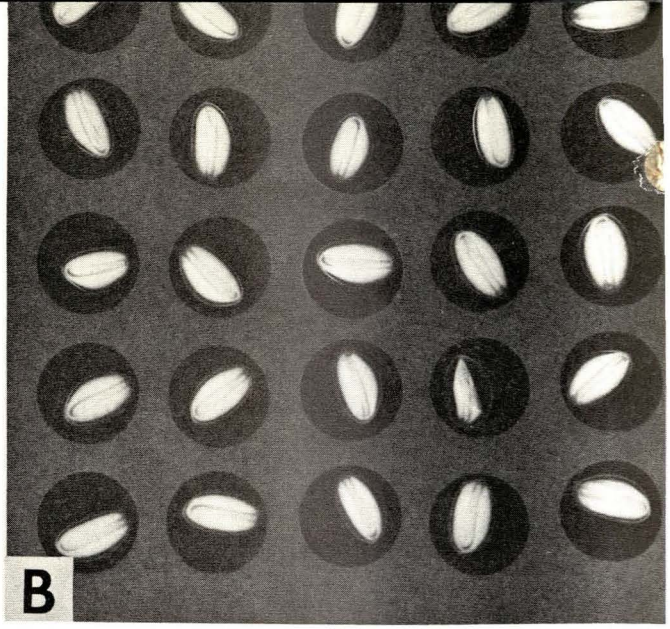
The quality of white pine hybrid seed, as judged from radiographs, has frequently been better than the quality of seed from crosses within the species *Pinus strobus*. As in *P. strobus* selfs, in hybrids we commonly find an "all or none" response: well-developed seed, or empty seed. Figure 6B is an example. This is filled seed of a cross of *P. strobus* x *monticola*. All embryos are well-developed, although one seed has an imperfect endosperm. Germinative capacity was very high in this case, although in some hybrid seed lots germinative capacity is low in seed that looks normal and well-developed.

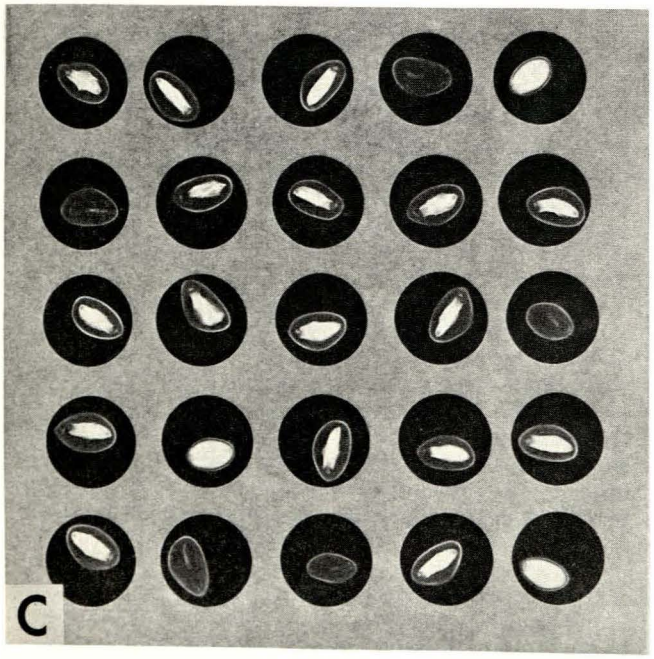
Figure 6C illustrates an unusual condition in an intraspecific *Pinus strobus* cross. The embryos were developing normally, but in most seeds the endosperm degenerated. In other seed lots from the same mother tree but different fathers, this condition did not exist. However most of these other seed lots did have some seed with incompletely developed endosperm. One cross with this mother tree produced very small seed.

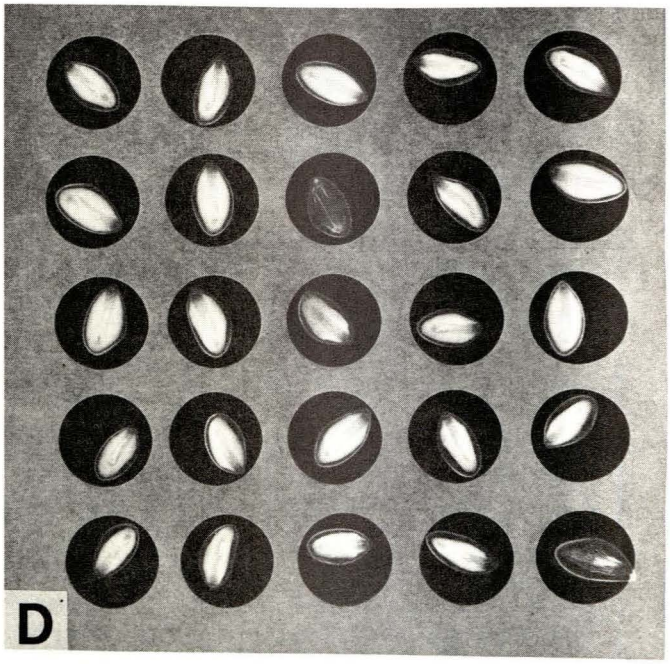
Seed from trees growing in a severe climate, as found at high altitude near timberline or at extreme northern limits of the species range, may be immature at the end of the growing season. The seed of *Pinus aristata* shown in figure 6D is an illustration. Not a single embryo is fully developed, and nearly all seeds have multiple embryos. Yet the seeds germinated rapidly and had a high degree of germinability after 90 days of stratification. Evidently embryo development continued during stratification. Simak and Gustaffson (1959) reported similar results from stratification of immature seed of *Pinus sylvestris*.

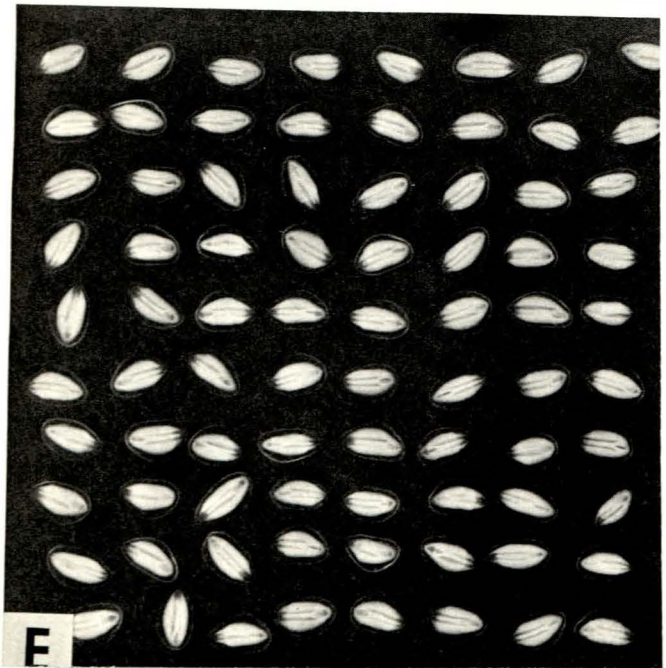
As a last example, we can compare two 10-year-old seed samples of *Pinus lambertiana* from two different trees, each of a different provenance. The seed in figure 6E was collected from a tree at an elevation of 6,000 feet in Eldorado County, Calif., and the seed in figure 6F from a tree at an unknown elevation in the Sequoia National Forest.

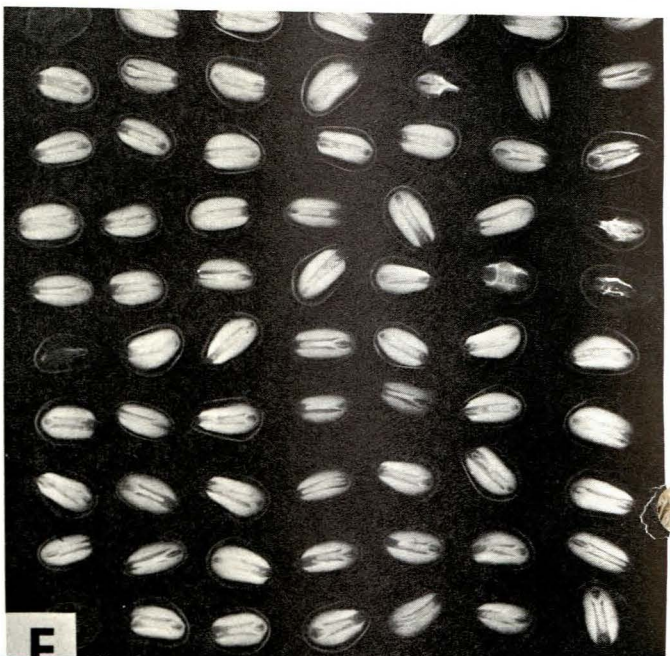

A


B


C


D


E


F

The seeds in figure 6F were significantly heavier than the seeds in figure 6E, although the radiograph shows that many of them are polyembryonic. Some have grayish embryos which are probably necrotic. The radiographs suggest that it might be advisable to X-ray seed samples from mountainous regions before including them in provenance tests. Comparisons of trees grown from two such dissimilar seed lots may be justified, but at least it would be desirable to know that such an internal condition exists.

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FIGURE 6. — Examples of pine seed radiographs. A, *P. strobus* x *strobus* (1513, 687 x 1281); B, *P. strobus* x *monticola* (1426, 1278 x 625); C, *P. strobus* x *strobus* (1509, 685 x 1281); D, *P. aristata* (1550, Hoosier Pass, Colo.); E, *P. lambertiana* (1455, Eldorado Co., Calif.); F, *P. lambertiana* 1456, Sequoia N. F., Calif.).

# CONE AND SEED YIELDS IN WHITE SPRUCE SEED PRODUCTION AREAS

by John A. Pitcher[1]

## Seed Production Areas

The source of seed is an important consideration in the reforestation program on the National Forests in the North Central Region. Thirty-five seed production areas have been set up in the Region, along the lines proposed by the North Central Forest Experiment Station, to provide control of seed source. Red pine, white pine, shortleaf and loblolly pine, and white spruce areas have been established. Including isolation zones, 1,500 acres are involved. Anticipated average annual yield is about 2,000 pounds of cleaned seed.

Nine of these seed production areas are established for white spruce. During the autumn of 1964, cone collections were carried out in four of them and cost records carefully kept. Table 1 summarizes the yields and costs.

## Cost of Seed

White spruce seed production areas on National Forest lands are set up with an average anticipated life of 20 years. Average cost of establishment runs about $250 per acre. This includes selection of area, marking, road construction, signs, supervision, and overhead costs. Compounded at 4 percent for 20 years this investment would yield $547.78. Prorated amortization is $27.39 per acre per year. Annual maintenance costs for roads, brush, insect and disease control, etc., run about $8 per acre per year. Total amortization is therefore $27.39 + $8.00 = $35.39 per acre per year.

Average seed yield is expected to be 2.4 pounds per acre per year. Each pound of seed would therefore carry the investment and maintenance costs of operating the seed production area. This amounts to $14.75. To this is added the cost of collection and extraction, which amounted to $28.34 per pound in 1964. The total cost of tree seed from white spruce seed production areas for the 1964 collection was $43.09 per pound.

The average cost of cleaned white spruce seed obtained from buying cones in the fall of 1964 was $9.84 per pound. Seed from white spruce seed production areas was costing us $33.25 more per pound, or about 3½ times the cost of buying cones from general collections.

## Cost of Planting Stock

Anyone who has had nursery experience realizes that the cost of seed is a minor part of the cost of producing planting stock. But just what was this "expensive" seed doing to our stock costs? Could we afford to continue this program?

Referring to table 1, we find that seed production area seed gives us more pure, live seed per pound. This means fewer pounds of seed are needed to yield any given number of seedlings. An analysis of the seed cost per thousand plantable seedlings shows the following:

[1]*Regional Geneticist, Region 9, Forest Service, U.S. Department of Agriculture, Milwaukee, Wis.*

Table 1.--Yield and cost comparison for collections from seed production areas and general collections, 1964

| Seed production area | Cone yield | Clean seed | Yield | Cost/lb. clean seed | Adjusted cost/lb.[1/] | Plantable seedlings/lb.[2/] | Seed cost per thousand | Stock cost per thousand |
|---|---|---|---|---|---|---|---|---|
| | Bushels | Lbs. | Lbs./bu. | Dollars | Dollars | Thousands | Cents | Dollars |
| Oconto River | 12.0 | 14.8 | 1.2 | 29.12 | 43.87 | 144.7 | 30.3 | 24.39 |
| Gates Lake | 32.0 | 39.4 | 1.2 | 33.83 | 48.58 | 176.4 | 27.5 | 24.36 |
| Watersmeet | 10.5 | 10.7 | 1.0 | 47.65 | 62.40 | 160.3 | 38.9 | 24.48 |
| Gilfillan | 16.0 | 24.8 | 1.6 | 10.83 | 25.58 | 126.3 | 20.2 | 24.29 |
| Total or (average) | 70.5 | 89.8 | (1.3) | (28.34) | (43.09) | (155.4) | (27.7) | (24.37) |
| General collection | | | | | | | | |
| Superior Nat. Forest | 98.5 | 71.1 | .7 | 11.52 | 11.52 | 146.1 | 7.9 | 24.09 |
| Chippewa Nat. Forest | 305.0 | 262.9 | .9 | 9.39 | 9.39 | 142.9 | 6.6 | 24.09 |
| Total or (average) | 403.5 | 333.9 | (.8) | (9.84) | (9.84) | (144.4) | (6.8) | (24.09) |

1/ Actual cost of collection and extraction plus annual prorated investment costs. No adjustment for general collections.

2/ (0.746)(purity x germination x no. seeds per pound) = number plantable seedlings per pound.

144,000 seedlings at $9.84/lb.
=6.8¢ per thousand
155,000 seedlings at $43.09/lb.
= 27.7¢ per thousand

The resultant increase in the cost of planting stock produced from seed production area seed is 21¢ per thousand.

## Program Cost

The average selling price for the 3,620,000 white spruce seedling transplants produced in our nurseries for the 1964-1965 planting season was $24.09 per thousand. The use of seed production area seed would increase the cost 21¢ per thousand to $24.30. This would represent a total program cost increase for white spruce of $756 for a production of 3,600 thousand.

Putting it another way, the use of seed collected from our seed production areas would increase the planting program cost by less than one percent. Using our 1964-1965 regional average of 750 trees per acre, we are investing 16¢ per acre of planting to obtain improved stock.

## Cost of Direct Seeding

The use of seed production area seed in direct seeding programs produces a more drastic cost difference than in the planting program.

Generally, about one-half pound of white spruce seed is required to sow one acre of forest land. At $43.09 per pound for seed production area seed vs. $9.84 for general collection seed, we can expect to increase the average cost of direct seeding by $16.62 per acre. Cost of seed treatment, site preparation, application of seed, and overhead would be about the same for general collection seed or seed production area seed.

In this situation, our seed costs very closely approach our stock costs on a per-acre basis. The only advantage lies in the lower cost of applying the seed in comparison to the cost of planting trees.

## Benefits

We spent $756 last year over and above previous production costs as a result of operating white spruce seed production areas. This is our total net investment in this phase of our white spruce tree improvement program.

What are we buying with this $756? Several things:

(1) We now have a positive geographic source. Not only can we pinpoint the source; we can also identify the broad climatic zone for planting and seeding. If we are willing to accept the early results of provenance tests now being conducted, we can expect better growing stock.

(2) We have made a phenotypic selection of parent trees. Whatever slight improvement we can anticipate in the way of increased growth rate and better form will tend to offset our investment and operating costs. A modest overall increase of only one-fourth of one percent in volume growth would mean an increase of $500 in total merchantable value.[2]

(3) The seed production area has a residual value when we no longer have use for it. A timber sale can be made in the area to harvest the sawtimber, piling, and pulp. The net value received for these timber products, if applied to our initial establishment costs, would also reduce our total investment.

(4) There are certain intangible benefits which we realize from using seed production area seed. Some nurserymen indicate that such seed gives better seedbed densities and more vigorous stock. Better initial survival of field plantings is also indicated, resulting in improved stocking of planted acres.

## Conclusion

While initial establishment and operating costs for white spruce seed production areas may seem high, analysis of the total program costs in relation to benefits received indicates a sound investment. Cone and cleaned seed yields from white spruce seed production areas are consistently higher than those from general collections. Knowledge of seed source and parent phenotypes provides for greater confidence in forest management planning.

The cost of collecting cones from standing trees is the largest single item in the operation of our seed production areas. We are now working on reducing this cost item by developing our own experienced cone collection crews, by contracting for the collection job, and by trying out new equipment and ideas.

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[2]*Figuring 0.8 cord per acre per year growth and a $6 per cord selling price for 4,000 acres of annual planting.*

# MASS PRODUCTION OF SHORTLEAF X SLASH PINE HYBRIDS BY POLLINATING UNBAGGED FEMALE FLOWERS

by Philip C. Wakeley, O. O. Wells, and T. E. Campbell[1]

A simple method of mass-producing shortleaf x slash pine hybrids was attempted. Large quantities of slash pine pollen were spread on unbagged shortleaf flowers in the hope of recovering a high percentage of hybrid progeny and thus materially reducing the cost of producing such hybrids by conventional controlled pollination. The hybrid is very promising, but, as Snyder and Squillace (1965) have shown, seed yields from controlled pollinations have averaged only 8.5 sound seeds per cone.

## Methods

The shortleaf pines were 10 natives in the Hodges Gardens and Experimental Area, Sabine Parish, Louisiana. They were open-grown, between 24 and 38 feet tall, and were flowering prolifically. One or more other shortleaf trees were within easy pollen flight of each.

The slash pine pollen was a mix of 1960 collections from several planted and native trees in central Louisiana and south Mississippi. Germination tests proved it highly viable. In April 1960, when the flower buds were large, examinations were made at intervals of 1 to 3 days to determine the optimum pollination time. When about one-third or more of the female flowers on a tree were receptive, the first application was made. The trees showed distinctive patterns of flowering; some matured the majority of their flowers almost simultaneously, others had flowers in several different stages at each re-examination and never had a large percentage receptive at one time. Thus, each tree was mass-pollinated from 1 to 4 times, depending upon the number of female flowers that were receptive at any one time.

The pollen was applied with an insecticide duster equipped with a 20-inch removable extension and an adjustable fishtail nozzle. The operator began at the top of the tree and worked downward. The duster held 75 cc of pollen and was easily reloaded in the tree. It was possible to cover practically the entire accessible portion of any of the 10 trees with 150 cc of pollen in an average time of 10 to 11 minutes. Tips of a few of the longest branches were missed, particularly when there was much wind. Missed branches were tagged so that they could be identified when cones were collected. To avoid wind, most of the work was done between 6:00 and 9:00 a.m.

Controlled pollinations with the same slash pine pollen were made on each tree to provide check material of known hybridity.

In the fall of 1961 all cones from controlled pollinations, and 40 cones from throughout the mass-pollinated portion of the crown of each tree, were collected. Seed was extracted and a sample from each lot was sown in the Harrison Experimental Forest Nursery near Gulfport, Miss., in the spring of 1962. The controlled- and mass-pollinated seeds from each tree were sown in adjacent rows to permit comparison with minimum effects of variation in nursery soil. At time of lifting, the seedlings resulting from mass pollination were separated into three groups per parent tree: putative hybrid, putative shortleaf, and undetermined. Those from each tree were classified by comparing them with their controlled-pollinated checks. Length, thickness, and straightness of needles, overall dimensions, and presence or absence of basal stem crook were the more important characteristics for classification.

The three groups of seedlings were then planted in the field, the mass-pollinated seedlings and the controlled-pollinated hybrid checks from the same female parent being placed in adjacent rows to facilitate final determination of hybridity.

## Results

After 2.5 years in the field, an average of 10.7 percent of the seedlings resulting from mass pollination showed definite evidence of hybridity (fig. 1 and table 1). Differences in numbers of hybrids among the individual-tree progenies were analyzed by chi square and were significant at the 1-percent level.

Practically all the hybrids that were evident in the field were in the group classified as hybrid in the nursery. During the first year in the field, however, it became clear that many non-hybrids had been included in the hybrid group. To improve classifying techniques, surplus seed from the study was sown in 1963 and the seedlings classified after one growing season in the nursery. Fifteen percent were judged to be hybrids, an estimate in fairly good agreement with the field result of 10.7 percent. It appears, therefore, that with some experience nursery separation of shortleaf x slash pine hybrids is practical.

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[1]*Principal Silviculturist (retired), Plant Geneticist, and Associate Research Forester, respectively, Southern Forest Experiment Station, Forest Service, U. S. Department of Agriculture. The study was conducted cooperatively with A. J. Hodges Forest Products Co., Inc.*

Table 1.--Estimation of number of shortleaf (female) X slash (male) pine hybrids in individual-tree progenies produced by artificial pollination without bagging female flowers

| Parent tree | Seedlings in progeny | Designated hybrid after 2.5 years in field. | |
|---|---|---|---|
| | Number | Number | Percent |
| A | 59 | 1 | 1.7 |
| B | 78 | 16 | 20.5 |
| C | 56 | 6 | 10.7 |
| D | 79 | 17 | 21.5 |
| E | 58 | 7 | 12.1 |
| F | 50 | 4 | 8.0 |
| G | 44 | 3 | 6.8 |
| H | 83 | 1 | 1.2 |
| I | 54 | 4 | 7.4 |
| J | 75 | 9 | 12.0 |
| Mean | 63.6 | 6.8 | 10.7 |

The mass-pollinated cones yielded an average 13.2 full seeds, the controlled-pollinated cones 6.6. As 10.7 percent of the mass-pollinated seeds were hybrids, an average of about 1.4 hybrids (10.7 percent x 13.2) were produced from each mass-pollinated cone, versus 6.6 hybrids from each controlled-pollinated cone.

While comparative costs were not determined, the mass-pollinated cones required from one to four trips to each tree for pollination plus a total of about 20 minutes' climbing time per tree. This probably would compare favorably with the cost of producing equal numbers of hybrids by controlled pollination. In addition, the present results indicate that the mass-production method can be improved, either by selecting shortleaf parents for compatibility with slash pine, by culling non-hybrids in the nursery, or by a combination of both.

If mass pollinations were carried out only on trees like B and D (table 1), which yielded 20 percent hybrid progeny, the technique would certainly be more economical than controlled pollination.

The cost of nursery-culling non-hybrids could be eliminated if hybrids and non-hybrids were planted together. As Righter (1946) has pointed out, planting a mixture of hybrids and of a species considered well adapted to the planting site would test the performance of the hybrids and at the same time assure the landowner of an adequate stand even if the hybrids failed. If the hybrids outperformed the non-hybrids, they would eventually comprise most of the crop trees in the mature stand even if they had originally been in the minority.

FIGURE 1. — Shortleaf x slash pine hybrid (left) and shortleaf pine (right), both from same female parent, after 2.5 growing seasons. Note the long, straight needles and heavier branches of the hybrid.

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Snyder, E. B. and A. E. Squillace. 1965. Cone and seed yields from controlled breeding of southern pines. U. S. Forest Serv. Res. Paper SO-21. Southern Forest Exp. Sta., New Orleans, La.

# STIMULATION OF EARLIER FLOWERING AND SEED PRODUCTION IN JACK PINE SEEDLINGS THROUGH GREENHOUSE AND NURSERY CULTURE

by Thomas D. Rudolph[1]

Attempts are continually being made to reduce the time between generations in forest trees through flower induction and stimulation studies and by selection for precocious flowering.

The pines have been of primary interest in research on early flowering. Righter (1939) surveyed the minimum ages of flower production among the pines grown at Placerville, Calif. The average minimum age for 39 species was 4.4 years for staminate strobili production; and for 55 species it was 5.2 years for ovulate strobili production. Jack pine (*Pinus banksiana* Lamb.) was reported as producing both male and female strobili at 3 years of age. More recently precocious flowering in pines has been described by Mergen and Cutting (1957), Reines and Greene (1958), Greene and Porterfield (1962, 1963), and others. Wright (1964) has summarized the information on flowering age in forest trees. In the pines he found earliest flowering in the *Lariciones* and *Insignes* series. Jack pine is a member of the *Insignes* group. Wright (1964) notes that jack pine seedlings grown on a wide spacing in a well-watered nursery can be expected to produce flowers by 3 years of age. However, the proportion of trees normally flowering at this age is extremely small. An examination of 100,000 three-year-old jack pine seedlings in standard nursery beds in Lower Michigan, but growing at a closer spacing than that described by Wright, showed that only approximately 0.3 percent of the seedlings bore first-year cones. Staminate strobili occurred with about equal frequency.[2]

The available evidence in the literature suggests, therefore, that early flowering or flowering before the age of 3 or 4 years in the pines is rare. However, as Wright (1964) points out, experiments indicate a possibility of reducing the flowering age of seedlings considerably by appropriate fertilizer and cultivation treatments.

Results given in this paper indicate that the flowering age in jack pine can be reduced and the proportion of flowering seedlings increased through cultural treatments.

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[2]*Rudolph, T. D. 1962 Unpublished report on file at the Institute of Forest Genetics, Rhinelander, Wis.*

## Materials and Methods

In June and November 1961, jack pine seed from three sources was collected: (1) 90 individual trees grown from X-rayed seed on the Argonne Experimental Forest in northern Wisconsin, (2) a bulked lot from a control plot of trees grown from nonirradiated seed, and (3) a stand of mixed plantation and natural origin on the Argonne Experimental Forest. The origin of (1) and (2) was the Chippewa National Forest in Minnesota. The seed was extracted from the cones immediately after each collection. Although the seed collections were made for another study (Rudolph 1966),[3] the observations reported here were made on the same plant material.

The first sowing was made on June 29, 1961, in fine sand on a greenhouse bench. Each of the individual tree collections was represented by a single 16-inch row containing 100 seeds. The control populations were represented by 4,500 seeds similarly arranged in 100-seed rows. The sand in the greenhouse benches was treated with a Tersan fungicide solution as required to check damping-off in the young seedlings. Commercial 10-10-10 fertilizer was applied as necessary to maintain the plants in a vigorous condition. A 20-hour photoperiod was maintained throughout the greenhouse phase of the study. Temperatures in the greenhouse were normally 75° F. during the day and 60° F. at night. However, day temperatures occassionally exceeded 75° F., especially in the summer and early autumn, and reached 90 to 95° F. on hot, clear days. During the summer and until about October 1, a 50-percent-shade saran cloth covered the greenhouse.

When the seedlings were from 2 to 3 months old, the following groups were transplanted into 2½ inch clay pots: (1) a random sample of 6 plants each from 53 of the 90 individual tree progenies, (2) a random sample of 120 plants from the nonirradiated control, (3) 160 plants from the natural stand collection, and (4) 50 plants in each of the cotyledon-number classes from 3 to 6 from the natural stand collection. Also, 17 seven-cotyledon plants were potted.

[3]*Also Rudolph, T. D. The effects of X-irradiation of seed on $X_1$ and $X_2$ generation progeny in Pinus banksiana. Ms. in preparation.*

A second sowing was made on December 15, 1961, following the same arrangement as that in June. Greenhouse conditions were the same, except that day temperatures did not reach the high levels experienced occasionally with the first sowing. When the seedlings were about 3 months old, a random sample of 6 seedlings from each of the 37 progenies not previously sampled was potted into 4-inch clay pots; this completed the potting of all 90 progenies being studied. All potted seedlings from this and the earlier sowing were subsequently grown under similar greenhouse temperature and photoperiod conditions. A commercial 10-10-10 fertilizer was applied to the pots as needed and the pots were watered daily.

Late in June 1962 the seedlings were transplanted into the nursery at a spacing of 10 x 10 inches. The seedlings with known cotyledon numbers were planted into 5 replications of 10 trees; all other seedlings were planted in identified row plots. A 50-percent-shade saran cloth covered the seedlings in the nursery bed for the first 6 weeks. The seedlings were fertilized in the nursery as required to maintain foliage color and vigor.

In late May and early June 1963, flowering was scored for each tree. At this time the older seedlings were 23 months and the younger group 17 months old. All seedlings in the individual tree progenies bearing female strobili were bagged for isolation and subsequent backcrossing to the female parent. A small number of flowering plants in the control and cotyledon-number groups were also bagged for subsequent controlled pollination. All other flowering seedlings were open to wind-carried pollen. Cones from the controlled pollinations and from the cotyledon-number population were collected in September 1964. The seed was extracted by hand and counted. Germination tests were made during the 1964-65 winter in the greenhouse.

## Results and Discussion

The results in table 1 show that female strobili production was stimulated in jack pine seedlings grown under the conditions described above. The large number of seedlings with female strobili (62

Table 1.--Female flowering and seed production on 17- and 23-month-old[1/] jack pine seedlings

| Date sown and seedling type | Trees observed: Number | Trees observed: Percent with female strobili | Avg. no. female strobili per tree | Percent of cones maturing | Avg. seeds per cone | Percent seed germination |
|---|---|---|---|---|---|---|
| June 29, 1961 | Section A. In relation to seedling cotyledon number: | | | | | |
| 3 cotyledon | 50 | 40.0* | 1.75 | 50.0 | 25.1 | 87.2 |
| 4 cotyledon | 49 | 65.3 | 2.19 | 54.3 | 26.1 | 76.2 |
| 5 cotyledon | 50 | 72.0 | 2.14 | 48.1 | 24.9 | 70.7 |
| 6 cotyledon | 47 | 66.0 | 2.16 | 47.8 | 30.3 | 79.7 |
| 7 cotyledon | 16 | 75.0 | 2.08 | 56.0 | 23.4 | -- [2/] |
| Total or mean | 212 | 61.8 | 2.08 | 50.5 | 26.4 | 77.0 |
| Dec. 15, 1961 | Section B. In X-irradiated individual tree progenies and control populations: | | | | | |
| 37-$X_2$ generation progenies[3/] | 216 | 23.1 | 1.34 | 10.4 | 25.9 | 35.6 |
| June 29, 1961 | | | | | | |
| 53-$X_2$ generation progenies[3/] | 309 | 41.4 | 1.80 | 29.0 | 26.6 | 53.3 |
| Non-irradiated control | 120 | 47.5 | 1.60 | -- | -- | -- |
| Mixed planted and natural stand | 151 | 56.5 | 1.32 | -- | -- | -- |
| Total or mean | 796 | 40.3[4/] | 1.56 | 24.8 | 26.4 | 51.4 |

* Percent of trees is significantly (5-percent level) lower than in other seedling types.

1/ Female strobili counted on June 1, 1963, when pollination was practically completed.

2/ Seed damaged during extraction; no germination data available.

3/ Seed yield and quality from these seedlings are based on results of controlled backcrosses to the female parent. Seed from all other seedling types was from open-pollinations.

4/ The mean for the seedlings sown on June 29, 1961, in Section B is 46.7 percent.

and 47 percent, respectively, in two lots of 23-month-old seedlings) contrasts sharply with 0.3 percent flowering on seedlings 1 year older grown under normal commercial nursery conditions. Noteworthy but unexplainable from the results of this study is the significantly smaller proportion of trees bearing female strobili in the 3-cotyledon seedlings. The decreased flowering in this group is apparently not related to height differences since there was no significant difference in height development between seedlings of the various cotyledon classes.

More than one-half of the female strobili produced developed into mature cones under wind-pollination conditions. The nearest pollen source was in a windbreak planting more than 50 yards from the seedlings in the transplant bed. In the controlled pollinations the proportion of female strobili developing into mature cones was reduced to slightly over 10 percent in the 17-month-old seedlings and 29 percent in the 23-month-old plants. Several factors may in part account for this. First, some of the female strobili may have been beyond the stage of receptivity before pollen from the female parents was available and applied. Secondly, some heat injury to the seedlings was noted due to temperature buildup within the cellulose casing-kraft paper double isolation bags. Further desiccation and injury to the developing strobili appeared to occur immediately following bag removal as a result of hot, dry weather conditions. Thirdly, the possibility cannot be discounted that some of the backcrosses attempted were incompatible. Finally, the lower proportion of female strobili maturing in the younger (and smaller) of the two seedling lots may be explained by more severe heat injury in this group due to two things: a larger proportion of the seedling was enclosed in the pollination bags, and the isolation bags were closer to the ground where higher temperatures prevail.

Seed yields per cone were comparable in the open- and control-pollinated groups; both fell within the range of average seed yields per cone reported by Rudolf (1958). Seed viability, however, was much higher in the open-pollinated seed than in that from the backcrosses. The 77-percent germination for the open-pollinated seed is markedly higher than the 68-percent average for jack pine (U. S. Forest Service 1948). The relatively high germination is even more impressive, as it is based on the actual number of seeds collected including empty seeds. The lower viability in seed obtained from the backcrosses may be accounted for to some degree by incompatibility that can occur at various stages of seed development in crosses between closely related genotypes. The potential increase in homozygous lethals at this level of inbreeding no doubt also tends to decrease embryo and seed viability. Furthermore, heat injury within the isolation bags as described above also may have influenced female gametophyte development and thereby subsequent embryo and seed development.

Strikingly, male strobili production was almost totally absent on the seedlings observed in this study. Less than 1 percent of the seedlings produced male strobili. The lack of male strobili and the stimulation of female strobili production may have been influenced by the environmental manipulations that the seedlings were subjected to during the period of study. Although the factors responsible for this response cannot be identified from the results, nor was the experiment designed for this purpose, several possible influencing factors are suggested.

In this study the environmental factor most drastically changed from what would occur in nature was the photoperiod. Although flowering in the pines has been considered to be day-neutral, Larson (1961) presents evidence suggesting that in jack pine photoperiod cannot be ruled out as a factor influencing flower formation. We have noted that in jack pine a 20-hour photoperiod applied continuously from the time of seed germination results in practically continuous vegetative growth for a year or more with only short intermittent hesitation periods for primordia formation and bud differentiation. The seedlings in this study were in a stage of active vegetative growth when transplanted into the nursery and into the decreasing photoperiod occurring after the summer solstice. Since, during a normal growing season, male strobili initiation in the pines occurs several weeks or even months before the female flower bud (Mergen and Koerting 1957; Larson 1961), it is most possible that the plants when moved to the nursery were subjected to a photoperiod not suitable for male strobili initiation. Further experimentation would be necessary to test this hypothesis.

In addition to consideration of the possible influence of photoperiod on flower initiation, it is reasonable to speculate that the increased vegetative growth induced in the seedlings by the extended photoperiod, resulting in abnormally large seedlings relative to their chronological age, may be related to the increased female flowering. Nienstaedt (1961) has suggested that age in the chronological sense can hardly be a determining factor in early flowering. He further suggests that size of a seedling as it is related to the balance of assimilating tissue to total amount of meristematic area may have an imporant influence on early flowering. A significant increase in the relative amount of assimilating tissue in the jack pine seedlings grown under long photoperiods in this study was evident. Needles, for example, often developed to a length of 10 inches, whereas under natural environmental conditions the needles rarely exceed 2 inches in length. Perhaps the long photoperiod regime influenced the formation of

increased numbers of female strobili in this indirect manner, as well as more directly through its effect during a particular plant growth phase on flower primordia initiation.

Further progress in stimulating earlier flowering could be expected if we could determine accurately the exact stage in vegetative development and the specific time during the annual growth period during which both female and male strobili are initiated. The necessary environmental manipulations could be applied more effectively during such critical periods of flower primordia initiation.

## Conclusions

The data presented in this paper suggest that flowering in jack pine, a species normally flowering at a young age, can be obtained even earlier and in increased magnitude through simple environmental manipulation during the first year of seedling development. The results further show that the breeding of such young trees, at least on a population or family basis, is feasible.

Female strobili formation on as many as two-thirds of the seedlings in a 23-month-old population and on up to one-fourth of 17-month-old seedlings, resulting from merely providing good growing conditions in the greenhouse and nursery, offers improved possibilities for the forest geneticist and tree breeder to accelerate their programs. With the ability to reduce the time between sexual generations to less than three years (including the period required for seed maturation), the forest geneticist has in jack pine an "experimental tree" species that will permit genetics research progress approaching that now enjoyed by non-woody annual plant geneticists. And the tree breeder can more quickly test the feasibility and demonstrate the potential gain from a tree improvement program by choosing jack pine as the test species. Analysis of advance-generation progeny tests can be accomplished in this species through stimulation of early flowering in a fraction of the time normally required for most forest tree species.

Undoubtedly, stimulation of female flowering in jack pine on the majority of plants prior to 2 years of age, as described in this paper, is not the ultimate in what can be accomplished in stimulation of early flowering. Through more critical environmental control along with genetic selection for early flowering it is not unreasonable to expect that eventually the age for consistent formation of both male and female flowers can be reduced to 1 year in jack pine.

Although flowering age may be further reduced in the future, the present results strongly suggest that jack pine may be a wise choice as an experimental species for fundamental research on the genetic behavior of the pines. Furthermore, jack pine warrants careful consideration as a "pilot" species in a full-scale tree improvement program.

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# TEN-YEAR HEIGHT GROWTH VARIATION IN LAKE STATES JACK PINE

by James P. King[1]

Jack pine (*Pinus banksiana* Lamb.) is one of the major pulpwood producing species in the Lake States. It is found on a variety of sites, but its outstanding characteristic is its ability to make early rapid growth on dry sandy soils.

In 1951 the Lake States Forest Experiment Station and the University of Minnesota initiated a seed source study of Lake States jack pine. Federal, State, and private forestry agencies collected seed from 29 stands in Minnesota, Wisconsin, and Michigan. The seedlings were used to establish 17 permanent test plantations in the three States.

Several reports have already come from this study. Rudolf and Jensen (1955-1958) described first- and second-year survival and second-year height growth and causes of mortality in all 17 plantings. Stoeckeler and Rudolf (1956) described seed source differences in fall coloration in the Hugo Sauer Nursery at Rhinelander. Differences between sources in susceptibility to white-pine weevil on the Chippewa National Forest plantations in northern Minnesota were reported by Batzer (1961). Arend *et al.* (1961) summarized seed source differences in 5-year height growth and white-pine weevil incidence in three plantings in Lower Michigan. Rudolph (1964) reported on differences in lammas growth and prolepsis of selected origins in four Minnesota and two Wisconsin plantings. Seed source differences in needle cast infection in a southern Wisconsin and a western Upper Michigan planting were described by King and Nienstaedt (1965).

This paper describes the height growth at the end of 10 growing seasons in 11 of the 17 plantations.

## Methods

In 1951 seed was collected from 29 jack pine stands in Minnesota, Wisconsin, and Michigan. Each collection was made from dominant and codominant trees in a stand considered good for its locality.

In the spring of 1952, seed from all 29 stands were sown in both the General Andrews State Nursery at Willow River, Minn., and in the Hugo Sauer Nursery at Rhinelander, Wis.

In 1954 two-year-old seedlings were used to establish 17 test plantations throughout the three States. Seedlings from the General Andrews Nursery were used in the six Minnesota plantings and two western Wisconsin plantings, while seedlings from the Hugo Sauer Nursery were used to establish four Wisconsin and five Michigan plantations.

At each location a four-replicated, randomized, complete-block design was used. Each replication contained a square 64-tree plot of each experimental seed source plus one "local" seedlot of stock furnished by a commercial nursery in the same area as the test plantation. Because of shortages within various seedlots, substitution had to be made in several plantings. Nevertheless, there were still 26 sources common to all plantings. The location of these 26 sources is shown in table 1 and figure 1.

Table 1.--Seed source location data

| Seed source number | County | Degrees of- Latitude | Longitude |
|---|---|---|---|
| | MINNESOTA | | |
| 1589 | Cass | 47.40 | 94.35 |
| 1590 | Cass | 47.05 | 94.52 |
| 1591 | Itasca | 47.55 | 94.05 |
| 1592 | Lake | 47.75 | 91.23 |
| 1593 | Cook | 48.00 | 90.33 |
| 1594 | St.Louis | 48.03 | 92.42 |
| 1595 | Pine | 45.95 | 92.55 |
| 1596 | Pine | 46.30 | 92.93 |
| 1597 | Becker | 47.08 | 95.47 |
| 1600 | Cass | 46.75 | 93.97 |
| 1601 | Beltrami | 47.48 | 95.00 |
| 1602 | Itasca | 47.75 | 93.28 |
| | WISCONSIN | | |
| 1605 | Bayfield | 46.75 | 90.95 |
| 1606 | Forest | 46.02 | 88.93 |
| 1608 | Burnett | 45.92 | 92.03 |
| 1609 | Marinette | 45.28 | 88.00 |
| 1610 | Oneida | 45.75 | 89.93 |
| 1611 | Wood | 44.45 | 89.72 |
| | MICHIGAN | | |
| 1612 | Gogebic | 46.22 | 89.20 |
| 1613 | Ontonagon | 46.56 | 89.02 |
| 1614 | Alger | 46.24 | 86.62 |
| 1615 | Chippewa | 46.26 | 84.84 |
| 1616 | Manistee | 44.26 | 86.26 |
| 1617 | Ogemaw | 44.34 | 84.12 |
| 1618 | Alpena | 45.00 | 83.54 |
| 1621 | Luce | 46.60 | 85.38 |

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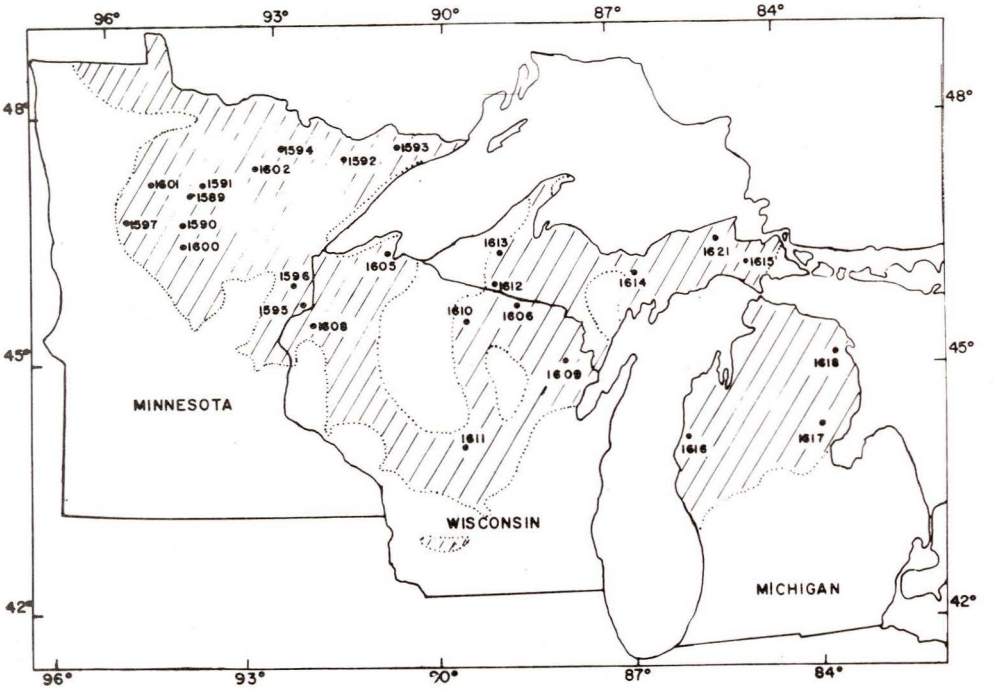


FIGURE 1 — Location of seed sources used in regional study. Shaded areas show main natural range of jack pine (Rudolf and Schoenike 1963).

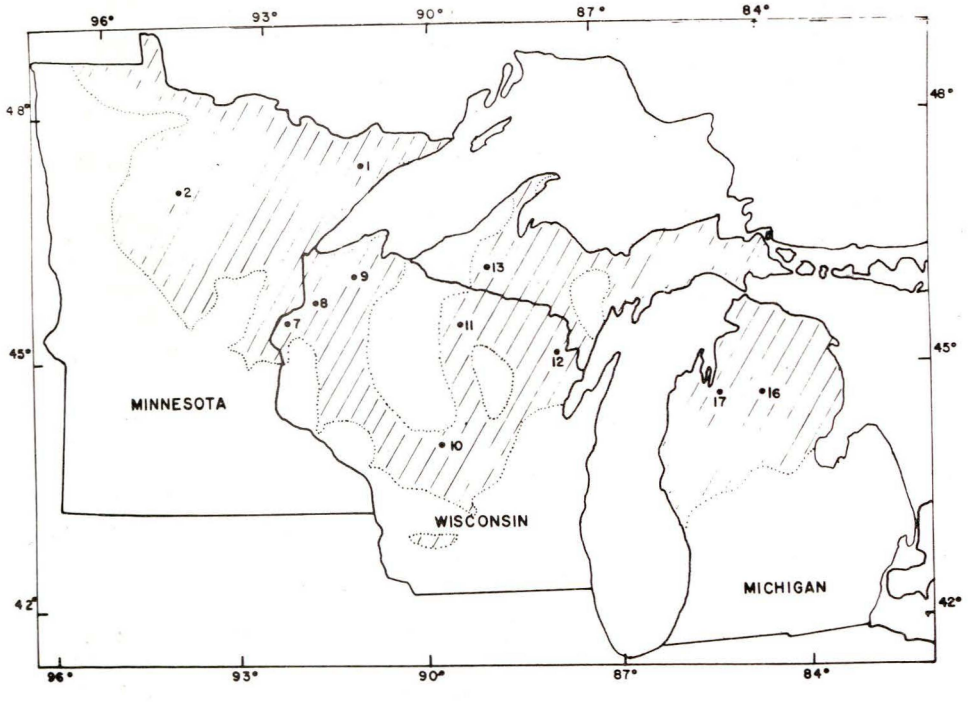


FIGURE 2. — Location of plantations measured in the fall of 1963. Shaded areas show main natural range of jack pine (Rudolf and Schoenike 1963).

In the fall of 1963, 11 of the test plantations (table 2, figure 2), then 10 years old, were measured for height, dbh, form, ramicorn branching, forking, and the presence or absence of seven insects and two diseases; 16 trees on each plot were systematically selected and measured.

Only the 10-year height (measured to the nearest 0.5 foot) of the 26 sources common to the 11 plantations and the local commercial seedlot will be presented here.

Table 2.--Location of jack pine test plantations measured in 1963

| Plantation number | County | Forest[1/] | Agency by which established | Degrees of- Lat. | Degrees of- Long. | Average second yr's. stocking (percent)[2/] |
|---|---|---|---|---|---|---|
| | | | MINNESOTA | | | |
| 1 | Lake | Superior NF | U.S. Forest Service | 47.6 | 91.4 | 97 |
| 2 | Beltrami | Chippewa NF | U.S. Forest Service | 47.4 | 94.6 | 88 |
| | | | WISCONSIN | | | |
| 7 | Burnett | Burnett CF | Burnett County | 45.6 | 92.8 | 93 |
| 8 | Washburn | Mosinee IF | Mosinee Pulp & Paper Mills Co. | 46.2 | 92.0 | 97 |
| 9 | Bayfield | Chequamegon NF | U.S. Forest Service | 46.3 | 91.3 | 92 |
| 10 | Wood | Nepco IF | Nekoosa-Edwards Paper Co. | 44.2 | 89.8 | 93 |
| 11 | Forest | Argonne EF | U.S. Forest Service | 45.7 | 89.0 | 98 |
| 12 | Marinette | Marinette CF | Marinette County | 45.6 | 88.0 | 95 |
| | | | MICHIGAN | | | |
| 13 | Ontonagon | Ottawa NF | U.S. Forest Service | 46.3 | 89.3 | 94 |
| 16 | Crawford | Au Sable SF | Michigan Conserv. Dept. | 44.5 | 84.7 | 97 |
| 17 | Grand Traverse | Fife Lake SF | Michigan Conserv. Dept. | 44.6 | 85.4 | 99 |

1/ NF = National Forest; CF = County Forest; IF = Industrial Forest; EF = Experimental Forest; SF = State Forest.

2/ Refers to percent of planted spots with thrifty trees (including replants) at end of second growing season (Rudolf and Jensen 1958).

## Results

The mean height of the plantation at 10 years in the field varied from 9.58 feet on the Chippewa National Forest in northern Minnesota to 15.15 feet on the Marinette County Forest in northeastern Wisconsin as shown in the following tabulation:

| *Plantation* | *Ave. height growth (feet)* |
|---|---|
| Minnesota | |
| Superior NF | 10.86 |
| Chippewa NF | 9.58 |
| Michigan | |
| Ottawa NF | 12.16 |
| Au Sable SF | 9.94 |
| Fife Lake SF | 10.39 |
| Wisconsin | |
| Burnette CF | 14.11 |
| Mosinee IF | 11.81 |
| Chequamegon NF | 11.90 |
| Nepco IF | 13.71 |
| Argonne EF | 12.75 |
| Marinette CF | 15.15 |

The six plantings in northern Wisconsin made the best growth averaging from 11.81 to 15.15 feet. The two plantings in northern Minnesota and the two in Lower Michigan were the four shortest plantings with means ranging from 9.58 to 10.86 feet.

There were significant seed source differences in 10-year height at every planting except the Chippewa National Forest. The sources ranged from a low of 25 percent below the plantation mean to 17 percent above the plantation mean (table 3).

At the two plantings in Lower Michigan and four of the Wisconsin plantings (Mosinee IF; Chequamegon NF; Nepco IF; Marinette CF), all three Lower Michigan sources (1616, 1617, and 1618) were in the five tallest sources. At the Burnette and the Ottawa NF plantings the five tallest sources included two from Lower Michigan.

In the two northern Minnesota plantations and one of the Wisconsin plantings (Argonne EF) the sources from north central Minnesota (1589, 1590, 1591, 1597, 1600, and 1601) did better as a group than the Lower Michigan sources (1616, 1617, and 1618). A combined analysis of data from these three plantings showed no significant seed source x plantation interaction. The Argonne planting is on a site that is colder and has a much shorter growing season than the immediately surrounding area. Thus it appears that the fast-growing Lower

Table 3.--Height growth of jack pine seed sources as a percent of the plantation mean

| Seed source | Superior NF** | Chippewa NF | Burnette CF** | Mosinee IF** | Chequamegon NF** | Nepco IF** | Argonne EF** | Marinette CF** | Ottawa NF** | AuSable SF** | Fife Lake SF** |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | MINNESOTA | | | | | | |
| 1593 | 104 | 96 | 94 | 79 | 88 | 90 | 88 | 90 | 90 | 94 | 95 |
| 1592 | 109[1/] | 97 | 93 | 77 | 83 | 91 | 99 | 91 | 89 | 98 | 98 |
| 1594 | 104 | 92 | 91 | 75 | 78 | 86 | 96 | 90 | 90 | 90 | 91 |
| 1602 | 105 | 99 | 95 | 88 | 88 | 95 | 103 | 93 | 97 | 95 | 98 |
| 1601 | 102 | 97[1/] | 105 | 105 | 103 | 98 | 105 | 101 | 100 | 103 | 105 |
| 1591 | 102 | 102 | 102 | 96 | 107 | 98 | 92 | 99 | 99 | 103 | 97 |
| 1589 | 101 | 102 | 99 | 99 | 107 | 106 | 102 | 105 | 106 | 103 | 103 |
| 1597 | 106 | 101 | 103 | 101 | 100 | 98 | 105 | 99 | 97 | 97 | 102 |
| 1590 | 98 | 103 | 103 | 105 | 98 | 99 | 98 | 102 | 105 | 106 | 99 |
| 1600 | 98 | 100 | 108 | 107 | 102 | 101 | 102 | 103 | 99 | 97 | 99 |
| 1596 | 104 | 107 | 105 | 108 | 104 | 104 | 104 | 100 | 99 | 99 | 101 |
| 1595 | 100 | 107 | 100 | 113 | 104 | 102 | 96 | 101 | 103 | 102 | 104 |
| | | | | | WISCONSIN | | | | | | |
| 1608 | 114 | 102 | 104[1/] | 110[1/] | 112 | 108 | 101 | 101 | 109 | 104 | 102 |
| 1605 | 90 | 100 | 97 | 99 | 103[1/] | 95 | 103 | 100 | 97 | 96 | 95 |
| 1610 | 100 | 100 | 103 | 102 | 100 | 105 | 108 | 105 | 105 | 104 | 105 |
| 1611 | 90 | 102 | 100 | 113 | 97 | 103 | 91 | 97 | 95 | 98 | 100 |
| 1609 | 92 | 101 | 102 | 108 | 101 | 104 | 98 | 102[1/] | 103 | 98 | 101 |
| 1606 | 99 | 94 | 95 | 92 | 100 | 93 | 101[1/] | 96 | 97 | 97 | 97 |
| | | | | | MICHIGAN | | | | | | |
| 1612 | 99 | 99 | 101 | 106 | 104 | 104[1/] | 105 | 97 | 106 | 96 | 100 |
| 1613 | 100 | 106 | 98 | 97 | 96 | 96 | 102 | 102 | 101[1/] | 99 | 93 |
| 1614 | 102 | 104 | 97 | 98 | 97 | 105 | 103 | 99 | 93 | 94 | 91 |
| 1621 | 94 | 92 | 94 | 90 | 93 | 92 | 94 | 95 | 97 | 95 | 92 |
| 1615 | 98 | 97 | 95 | 92 | 96 | 95 | 101 | 101 | 99 | 93 | 95 |
| 1616 | 98 | 98 | 109 | 117 | 116 | 116 | 102 | 109 | 109 | 115 | 113[1/] |
| 1617 | 93 | 97 | 110 | 111 | 109 | 110 | 97 | 114 | 106 | 110[1/] | 111 |
| 1618 | 99 | 105 | 105 | 114 | 108 | 105 | 105 | 106 | 109 | 116 | 113 |
| | | | | | LOCAL NURSERY | | | | | | |
| | 111 | 94 | 94 | 94 | 92 | 88 | 90 | 96 | 97 | 101 | 113 |

1/ Stand collection from nearest the planting site.
** F value shows a significant difference between at least two seed sources at the 1-percent level. (Tabular F .01 = 2.07 with 25 and 75 degrees of freedom.)

Michigan sources (1616, 1617, and 1618) are not suited to areas that are colder than the average northern Wisconsin temperatures.

In each of the six Wisconsin plantings and the planting in the western portion of Upper Michigan (Ottawa NF), the best test source exceeded the local commercial nursery stock by 12 to 28 percent in height growth. Only on the Superior NF in Minnesota and the Fife Lake SF in Michigan did the commercial nursery stock fail to differ significantly from the best source in the planting.

In every plantation in Wisconsin and in two of the three Michigan plantations the seed source collection from nearest the planting exceeded the commercial nursery stock in height growth (although in some plantings this difference was not significant). Since these seed source collections were from stands considered good for their area whereas the commercial nursery stock was from unselected stands, even relatively mild phenotypic selection may be worthwhile in this species.

When data from the 11 plantations were combined into a single analysis, there was a significant amount of seed source x plantation interaction (interaction variance/error variance F ratio = 2.61 with 250 and 750 degrees of freedom). The most notable source of this interaction was the change in height growth of the northern Minnesota (1592, 1593, 1594, and 1602) and Lower Michigan sources (1616, 1617, and 1618) between the Minnesota and Wisconsin-Michigan planting sites.

However, other sources contributed to the interaction as well. For example, when three Wisconsin plantings (Burnette CF; Mosinee IF; Chequamegon NF) and the planting in western Upper Michigan (Ottawa NF) are considered, the Lower Michigan sources (1616, 1617, and 1618) are the best group and the northeastern Minnesota sources (1592, 1593, 1594, and 1602) the poorest group, but there is still a highly significant amount of interaction present ($F = 2.37$ with 75 and 225 degrees of freedom). But since these interactions seldom involve the very best sources, they are of little practical consequence.

An interesting result of these interactions is their effect on correlations between seed source performance and seed source climatic and geographic factors. Were an experimenter computing correlations between 10-year height growth and seed source latitude based on the Lower Michigan plantations, he would find negative correlation ($r = -.60$ with 24 degrees of freedom). However,

were data from two northeastern Minnesota plantations used in the same computations, the correlations would be positive but nonsignificant ($r = .28$ with 24 degrees of freedom).

Moreover, were a researcher working only with data from the well differentiated Wisconsin and Michigan plantations, he might conclude that the jack pine in the Lake States was composed of a number of distinct subpopulations since the Lower Michigan sources (1616, 1617, and 1618), the Upper Michigan sources (1614, 1615, and 1621), and the northeastern Minnesota sources (1592, 1593, 1594, and 1602) all behave similarly within most plantings and still differ significantly from adjacent groups of seed sources.

However, were only data from the two Minnesota plantings plus the Argonne EF planting in northern Wisconsin considered, the researcher might conclude that the jack pine of the region was composed of a single population with an essentially random variation pattern.

This study thus emphasizes the difficulty in studying patterns of within-species genetic variation: Subpopulational differences may express themselves only where the growing conditions are especially favorable (or unfavorable) for a given subpopulation.

## Seed Collection Recommendations

The data in this study were collected on relatively young trees. Even so, certain useful seed collection recommendations can be made.

Where the superiority of local seed sources is indicated as in the Lower Michigan plantations, we can safely recommend the use of local seed from carefully selected better-than-average stands as a means of increasing early growth rate.

However, where the superiority of nonlocal sources is indicated, such as in the Wisconsin plantations, the data must be used with caution. In this case one might consider planting mixtures of both local and nonlocal stock. This would give the forest manager some immediate growth increase and still leave him protected against some catastrophic failure of the nonlocal stock in later years.

Specifically, the following recommendations seem reasonable and safe:

1. Collect seed only from better-than-average appearing stands.

2. In Lower Michigan plantings, use only seed collected in Lower Michigan.

3. In Wisconsin and Upper Michigan collect seed from Lower Michigan and mix the Lower Michigan seedlings with seedlings from local stands.

4. In Minnesota collect seed from selected stands near the planting site.


## Summary

In the fall of 1963, 10-year height was measured in the Lake States jack pine seed source study. Data on 26 seed sources from Minnesota, Wisconsin, and Michigan that were common to 11 test plantations in the three States are presented along with height growth of a local commercial nursery stock.

Seed sources from Lower Michigan performed best as a group throughout Michigan and Wisconsin. In the northern Minnesota plantings the group of sources from north central Minnesota did best.

At 9 of the 11 plantings the commercial nursery stock was shorter than the test sources by 12 to 28 percent. In almost all of the plantings the test seed source from nearest the planting outgrew commercial nursery stock (though not always significantly).

Whether one considers the variation pattern in jack pine to be continuous or discontinuous depends to some extent on the location and precision of the test plantations. However, in the most strongly differentiated plantings, the variation appears discontinuous.

The selection of "good" jack pine stands for seed collection (phenotypic selection) appears worthwhile in this species. However, selection of tested nonlocal stock will offer even greater improvement.


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# THE PROBLEM OF SITE VARIATION WITHIN RED PINE PROVENANCE EXPERIMENTS

by Mark J. Holst[1]

In spite of care taken in the selection of site and experimental design of provenance experiments, site heterogeneity within the experimental area may be more complex than was anticipated when the experiment was established. The present paper describes a problem of this nature encountered in a red pine (*Pinus resinosa* Ait.) provenance experiment at the Petawawa Forest Experiment Station. An appropriate design for minimizing this problem is proposed.

## Background

Red pine grows best on fertile sands with good aeration and moisture supply. On these soils, it is a fine straight tree of high economic value. It is not surprising that it has been widely planted both in Canada and the United States. Failure owing to planting on heavy soil has been reported (Stone *et al.* 1954, Richards and Stone 1964), but there is little information concerning red pine's reaction to fertility, drought, and frost (Wilde 1964).

Red pine shows a remarkable stability in taxonomic characteristics, and the stem form is uniformly good. One could, therefore, easily get the false impression that there are few important differences in the species associated with provenance. However, recent studies show clearly that physiological races do exist (Holst 1964b) although they are hard to distinguish morphologically, and the range in rate of growth is relatively limited.

The older red pine provenance experiments established in the Lake States (Lester 1964, Nienstaedt 1964, Rudolf 1964) and Pennsylvania (Hough 1952) were planted on moderately fertile and uniform sands in congenial climates. In these circumstances, survival was good, the trees appeared healthy, and reports about them mention no excessive variation because of soil heterogeneity. Differences among provenances were small, and none of the provenances was damaged by frost or the effects of drought.

In Canada where red pine provenance experiments have been planted near the northern limit of the species or on marginal soil, differences among provenances have been more evident (Holst 1964a). On the marginal soils red pine has reacted strongly to variations in microsite.

## Effects of Macro-Site Differences

Provenance experiments conducted on uniform, fertile, and weed-free nursery soil are better than tests planted on less uniform forest soils for providing estimates of differences among provenances. This was demonstrated in one of our oldest red pine provenance experiments.

Ten Ontario provenances were sown in 1951. A nursery provenance experiment was planted in 1952 with 25-tree plots and three replications. A field experiment was planted in 1955 on a somewhat frosty and variable site[2] with 100-tree (10x10) plots and three replications. Single division rows of Petawawa red pine were planted between the plots to aid the assessment of any site differences that might appear later. Thus 44 division row plants were associated with each plot.

A comparison of the 1954 heights in the nursery test with the 1957 heights in the field test is shown in figure 1. One particular provenance is clearly

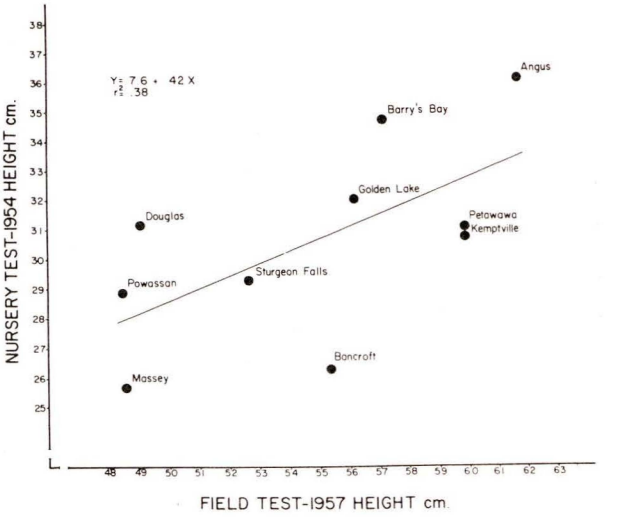


FIGURE 1. — Comparison of heights of red pine provenances in a nursery test on uniform soil and in a field test on variable soil.

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[2]*The test site was an old beach line with sand of variable texture and shallow frosty depressions. The mounds had better soil than the depressions. Thus there is a confounding effect of soil fertility and frost that could be mapped, but not removed by the experimental design. Mounds accounted for roughly 15 percent, depressions 60 percent, and intermediate 25 percent of the experimental area.*

best in both instances, but in general there is a great lack of consistency between the two plantings. The relationship between height and length of growing season for the areas of seed origin is significant in the nursery test ($r = 0.78^{**}$), but is not significant in the field test ($r = 0.62$). Duncan's Multiple Range Test at the 5 percent level of probability distinguished four groups in the nursery test, but only three groups in the field test. The F ratios for provenances were about the same in both experiments.

The 1959-60 winter was severe and was followed by a late spring frost that caused considerable injury to the red pine in these experiments. The opportunity was taken to study spring frost damage in relation to provenance. In the early summer of 1960, all trees in plots and surround rows were scored as follows:

| *Score* | *Degree of frost injury* |
|---|---|
| 1 | No damage. |
| 2 | No damage on upper part of the crown, but up to five branches nearer ground level browned by frost. |
| 3 | Less than 75 percent of the crown browned by frost. |
| 4 | More than 75 percent of the crown browned by frost. |

In viewing the experiment as a whole, it was evident that the intensity of frost injury was related to minor variations in elevation over the area of the experiment. The area was therefore stratified according to three general levels of frost intensity: heavy (low elevation), medium (intermediate elevation), and light (high elevation). Individual trees and provenances exhibited varying degrees of frost injury within each intensity level. Average scores of frost injury were calculated for each plot, and for subplots formed within plots by the boundaries of the general zones of frost injury. In view of the confounding effect of site variation due to elevation, the problem was to determine the component of variation in frost injury associated with provenances.

The analyses of variance among plot means, plot means adjusted for surround rows by covariance (deviation of particular surround rows from the overall surround row mean), and plot means in percent of surround rows, are listed in table 1. Differences among provenances based on observed plot means were not significant, but reached significance (5 and 1 percent) after the adjustments for surround rows were made. The error variances for the observed plot means and plot means adjusted for surround rows by covariance were directly comparable and indicated a threefold gain in precision.

These are standard procedures but they may give a false impression of the real pattern of injury. The relatively few plots occurring in the medium and light frost areas made it difficult to run separate regressions for each injury zone. Instead I plotted the frost damage relative to surround rows in the heavy frost area only against the plot means adjusted for surround rows by covariance (fig. 2). The scatter is considerable. Since it is considered that a truer relationship between frost damage and provenance is to be found in the area with heavy frost, the conclusion must be that the standard covariance methods for adjustment of plot means were inadequate, even when based on a grid of surround rows.

In this particular layout, mapping of the three levels of frost intensity gave a better rating of provenance differences than plot means adjusted for surround rows, which again were three and a half times better than comparisons of the unadjusted plot means. The ratio between plot plants and surround plants was nearly 2:1 (100:44). A more accurate estimate could be made by omitting the surround rows and instead establishing plot plants and check plants in a ratio of 1:1. I shall return to this problem later.

Table 1.--Analyses of variance of frost damage index

| Source | Observed plot means (C.V.= 18)[1/] | | | Plot means adjusted for surround rows (C.V.= 10)[1/] | | | Plot means in percent of surround rows (C.V.= 11)[1/] | | |
|---|---|---|---|---|---|---|---|---|---|
| | d.f. | M.S. | F | d.f. | M.S. | F | d.f. | M.S. | F |
| Total | 29 | | | 28 | | | 29 | | |
| Blocks | 2 | | | 2 | | | 2 | | |
| Provenances | 9 | 0.28 | 1.0 | 9 | 0.29 | 3.6* | 9 | 584 | 4.2** |
| Error | 18 | 0.28 | | 17 | 0.08 | | 18 | 138 | |

1/ C.V. = coefficient of variation = $100\frac{S}{\bar{x}}$ percent.
Significance levels: **, 1 percent; *, 5 percent.

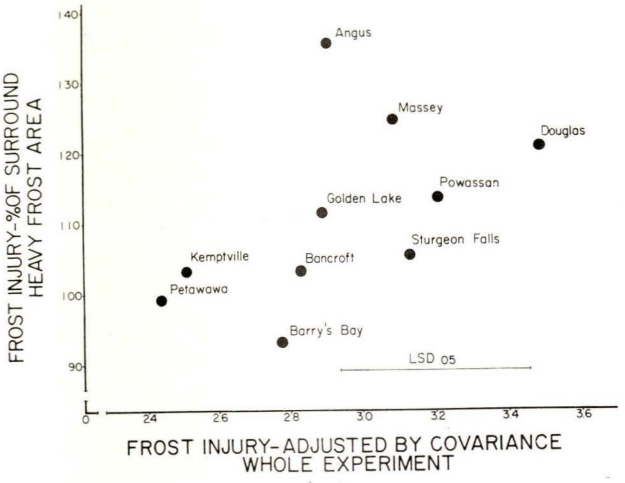



FIGURE 2. — Spring frost injury on red pine provenances. Comparison of spring frost injury relative to surround row (Petawawa provenance) in area of heavy frost, and index of spring frost injury adjusted for surround row performance by covariance.

In establishing experiments of standard design where there are no surround rows or check plants to serve as indicators of soil heterogeneity, the best way to deal with the excessive variability induced by important macro-site differences, is to prepare a map of the site differences. These differences are best judged on a basis that is independent of the experimental plants, such as soil characteristics or elevation. Even a crude separation can be helpful in applying meaningful adjustments that improve the interpretative value of the experiment. Where the macro-site differences are less pronounced we have found that it is possible to get acceptable results by measuring the dominant trees only.

## Effects of Micro-Site Differences

The other point I should like to demonstrate is the effect of micro-site on tree height and survival. In this red pine provenance experiment striking trends in tree height were observed within the area of the experiment. The trees were spaced at 4x4 feet. Within the space of four or five trees (i.e. 15 to 20 feet) one could see a gradient from tall trees to short or even missing (dead) trees. As this is an area of outwash sand and gravel one would suspect differences in moisture-holding capacity that would influence the survival and growth of the red pine. This has been demonstrated in red pine plantations on similar sites at the Petawawa Forest Experiment Station.[3] However, there also may be some soil factor which is poisonous to red pine roots.

During the summer of 1965, the soil was sampled along 16 tree height gradients. As the seedlings were planted in plowed furrows, samples were taken from the undisturbed soil at the bottom of the furrows. Tree heights were recorded along each gradient. The soil samples were set out in the greenhouse, watered with distilled water, and sown with red pine seed. Table 2 shows the relation between tree heights in the plantation and germination. It demonstrates delayed germination in the soil taken under trees with intermediate growth, and both delayed and low germination in the soil taken under the trees of stunted growth.

Trees grown under adverse conditions of moisture, nutrition, or gas exchange are more susceptible to injury by frost than trees grown under optimal soil conditions. In addition to the variability induced by these causes, there appears to be some factor which inhibits germination and

[3]*Several research workers at the Petawawa Forest Experiment Station have studied fail areas in red pine plantations. Some are of the opinion that certain fail areas are caused by antagonistic effects of sweetfern (Comptonia peregrina (L) Colt), sometimes associated with an attack by Saratoga spittlebug (Aphrophora saratogensis Fitch). A recent investigation by Dr. P. J. Rennie, head of the Soils Section, indicated that some fail areas are associated with a high silt content which creates a compact, poorly aerated rooting medium for red pine. It has also been noted that the poorly developed red pines in those areas were damaged by frost.*

Table 2.--Germination of red pine seed in soil taken along a deficiency gradient as indicated by tree height

| Tree height (cm) | Germination percent 1/ | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Date in August | | | | | | | |
| | 12 | 14 | 16 | 18 | 20 | 22 | 26 | 28 |
| 345 | 2 | 10 | 19 | 22 | 28 | 58 | 73 | 79 |
| 240 | 5 | 15 | 25 | 31 | 42 | 62 | 77 | 79 |
| 110 | 0 | 2 | 2 | 4 | 19 | 37 | 72 | 78 |
| 80 | 0 | 2 | 4 | 4 | 10 | 16 | 47 | 56 |

1/ Mean of two samples of 100 seed.

may also limit root development. In milder cases growth is retarded. In severe cases the plant is killed.

In an experiment with different seed-covering material we have noticed that washed gravel from our local gravel pit also resulted in delayed germination of red pine seed. The cause has not yet been identified.

Difficulties in experimentation arising from micro-site differences can be reduced by increasing the number of plants per plot and then measuring only the taller trees in each plot.

## Soil Heterogeneity and Experimental Error

Provenance experiments are designed in part to estimate population site interactions in yield. The purpose may relate directly to problems in plantation management; or it may be to provide estimations of genetic parameters essential for a program of selection and breeding. Experimental plots should be large enough to minimize error in yield estimates that may be induced by variations among plots in stand density, competition, site, or soil.

To keep the experimental error at a minimum the site chosen for experimental tests should be as homogeneous as possible. It is difficult to distinguish a homogeneous site because under natural conditions stand stratification may result from differences in age, site, competition, genotype, and their interactions. A mature stand of widely spaced trees may give a false impression of site uniformity which is dispelled when plantations are established at close spacing.

It has been shown that standard statistical designs are inadequate to account for site heterogeneity found in some forest experiments (Wright and Freeland 1959, 1960). This is a common situation. I have seen many provenance experiments in both Europe and North America in which, in spite of careful attention to experimental design, the level of precision has been low owing to marked soil heterogeneity. The precision of these experiments could have been increased by the inclusion of suitable checks on soil heterogeneity.

In the red pine experiment, the error variance was reduced by accounting for some of the site effect with surround row plants. Here the ratio of plot:check plants was approximately 2:1. The error would be further reduced and the sensitivity of the covariate increased if check plants were established in a 1:1 ratio with plot plants, as in the black and white squares on a checker board. Each plot plant could then be rated relative to four adjacent check plants.

The primary advantage of this intensive system of check and plot plants is that it permits the estimation of intra-plot as well as intra-block variation in levels of site productivity.

From this it follows that:

1. Plot size would not be critical in relation to soil heterogeneity.
2. Estimates of plot and block variation could be made independently of the plot plants.
3. Analysis would yield values for check x provenance x block interactions that estimate the stability of individual provenances within the experiment.
4. Extremely poor parts of the experiment could be objectively identified and rejected if necessary.
5. After the check trees have provided early estimates of site heterogeneity, they may be thinned or retained according to need.

If the main objective of the experiment is to compare exotic provenances with the local provenance, then the check trees should be from a selected population of local origin. In this case the most important comparison is made with a very high degree of precision.

The check plants should normally be of the same species as the plot plants. Seed for the check plants should be collected from natural stands rather than from plantations. The population of which the check plants are a sample should be chosen according to the objective of the experiment, e.g. a slow-growing type may be desirable. The check plants should be treated with the same care and attention in the nursery as the plot plants, and should be heavily graded for uniformity before being planted out. It may be desirable to include four different populations as sources of check plants to guard against specific population x environment interactions[4] (see fig. 3).

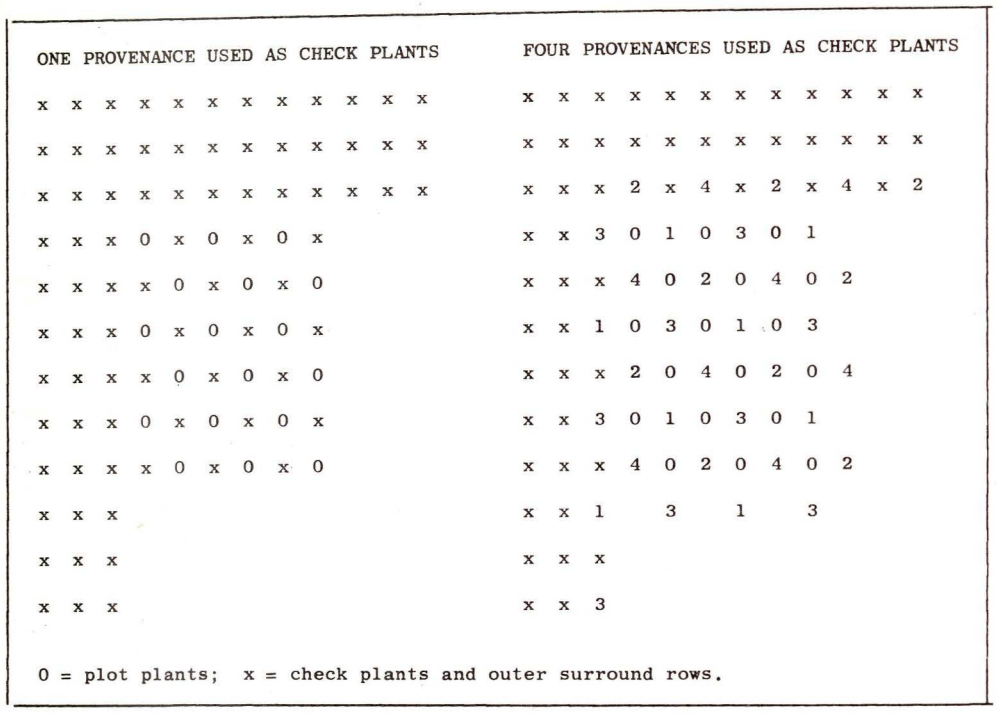
This method of accounting for environmental heterogeneity is proposed as a means of dealing with the common problem of controlling experimental error. It is my hope that the inclusion of check plants in suitable experiments will be tested by a number of investigators in the field of forest genetics.

## Summary

To demonstrate the effect of macro- and micro-site differences, a red pine provenance experiment planted on a variable site at the Petawawa Forest Experiment Station was analysed. By subdividing the area into three classes of frost intensity, and by expressing plot means relative to their surround rows, the error term was reduced by one-fourth of the error term obtained when the plot means were compared directly.

Variation in micro-site was also pronounced in this experiment. Soil seed germination antagonism was demonstrated from soil sampled from fail areas. Delayed germination of red pine seed occurred in the soil taken under trees with intermediate

[4] *Suggestion made by Dr. W. J. Libby at the Forest Genetics Workshop.*


ONE PROVENANCE USED AS CHECK PLANTS
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0 = plot plants; x = check plants and outer surround rows.

FIGURE 3. — Sample layouts of alternate planting of plot plants and check plants.

growth, and both delayed and low germination in the soil taken under trees with stunted growth. The cause of delayed or low germination was not identified.

It is suggested that a better estimation of the influence of soil heterogeneity could have been obtained by alternate planting of plot plants and check plants so any plot plant could be rated relative to the four adjacent check plants. The advantages of such a system are: plot size is not critical; size of experimental area is not a limiting factor (which is important when many provenances are tested); the estimation of population differences will be more precise; extremely poor parts of the experiment can be identified (and rejected if necessary); after the check plants have served their purpose as indicators of soil heterogeneity they can be removed if desired.

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# PINE POLLENS FROZEN FIVE YEARS PRODUCE SEED

by R. Z. Callaham and R. J. Steinhoff[1]

Deep-freezing of pine pollen offers a means of prolonging its storage life. Early work showed that pollen could be frozen without losing its viability. A study was started in 1958 at the Institute of Forest Genetics at Placerville to determine how long frozen pollen of several pines would remain viable. This paper reports *in vitro* germination and *in vivo* seed production by pollens stored at −20°C. up to 5 years.

References to low temperature storage of forest tree pollens are infrequent in the literature. Boden (1958) successfully stored eucalyptus pollen at −16°C. for 7 months. Duffield and Callaham (1959) reported success in freezing pine pollen for 1 year at −23°C. They found that fresh and stored pollen were about equally effective in producing sound seed. Wright (1959) mentions good results using pollen stored for 1 year at −18°C. Ehrenberg (1961) reported results of crossing experiments with pollen stored for 3 years in a freezer. Her *in vivo* studies with *Pinus sylvestris* showed that storage at +4°C. was much less effective than storage at −18°C. After 3 years, pollens stored at +4°C. and −18°C. both germinated nearly 100 percent *in vitro*. Crossings using the pollen stored at +4°C. did not produce any filled seed, but crossings using pollen stored at −18°C. gave only slight reductions in seed-set and produced about half as many filled seed as would be expected from fresh pollen.

## Methods and Materials

The study was initiated in 1958 with the following species: *Pinus jeffreyi* Grev. & Balf., *P. monticola* Dougl., *P. contorta* var. *murrayana* (Grev. & Balf.) Engl., *P. ponderosa* Laws., and *P. sabiniana* Dougl. For each species three trees that flowered early were selected to serve as pollen parents and three that flowered late were selected as seed parents. All trees were located on a transect through the Sierra Nevada near Placerville, Calif.

In the spring of 1958, ripe pollen was collected and extracted, using the technique described by Cumming and Righter (1948). Pollen moisture content was not controlled, but pollen was dried to the point where it flowed.

Pollen from each tree was divided into several small aliquots in small screw-cap glass bottles. Each aliquot contained enough pollen to pollinate the conelets in 9 pollination bags. One aliquot was stored at room temperature until used from 2 to 10 days later. Two aliquots were placed in a domestic refrigerator kept at about 5°C. to be used after 1 and 2 years of storage. Nine aliquots were placed in a domestic deep freezer kept at −20°C. The first aliquot from the deep freezer was used from 2 to 10 days later when female parents were ready to be pollinated. The remaining aliquots were to be used after 1, 2, 3, 5, 7, 10, 15, and 20 years of storage. In each test year, fresh pollen for comparison was again collected from the original pollen parents. All pollens were tested for viability by the method of Righter (1939). Pollens removed from storage were kept at room temperature until pollinations were completed.

Fresh and stored pollens were used in controlled pollinations (Cumming and Righter 1948) on each seed parent in 1958, 1959, 1960, 1961, and 1963. On a seed parent tree each pollen aliquot was used to pollinate the strobili isolated in three pollination bags. Ripe cones were collected, and seeds were extracted. Filled and hollow seeds were counted after separation by winnowing. A sample of hollow seeds was examined for insect damage. All infested seeds were presumed to have been filled, and numbers of filled and hollow seeds were corrected accordingly.

## Results and Discussion

All desired crosses to determine ability of stored pollen to produce seed *in vivo* could not be completed. Some species had sporadic cone production or very low seed set from controlled pollination. Squirrel depredations eliminated crosses on some species. One tree adorned with pollination bags was felled. Only the results for *P. monticola* were complete. The next best results were for *P. jeffreyi*. Only data for these two species warranted statistical analysis. Fragmentary data for the other three species are discussed wherever they provide useful information.

Brief freezing of pollen for 2 to 10 days immediately following extraction increased yield of filled seeds (figs. 1 and 2). For *P. jeffreyi* the increase was large and statistically significant; for *P. monticola* it was slight. In contrast, fragmentary data for *P. sabiniana* showed that fresh pollen gave

[1]*At the time this research was done, both authors were at the Institute of Forest Genetics, Pacific Southwest Forest and Range Experiment Station, Forest Service, U. S. Department of Agriculture, Placerville, Calif. Callaham now is Chief, Branch of Genetics, Division of Timber Management Research, U. S. Forest Service, Washington, D. C. Steinhoff now is at the Forest Science Laboratory, U.S.D.A. Intermountain Forest and Range Experiment Station, Moscow, Idaho.*

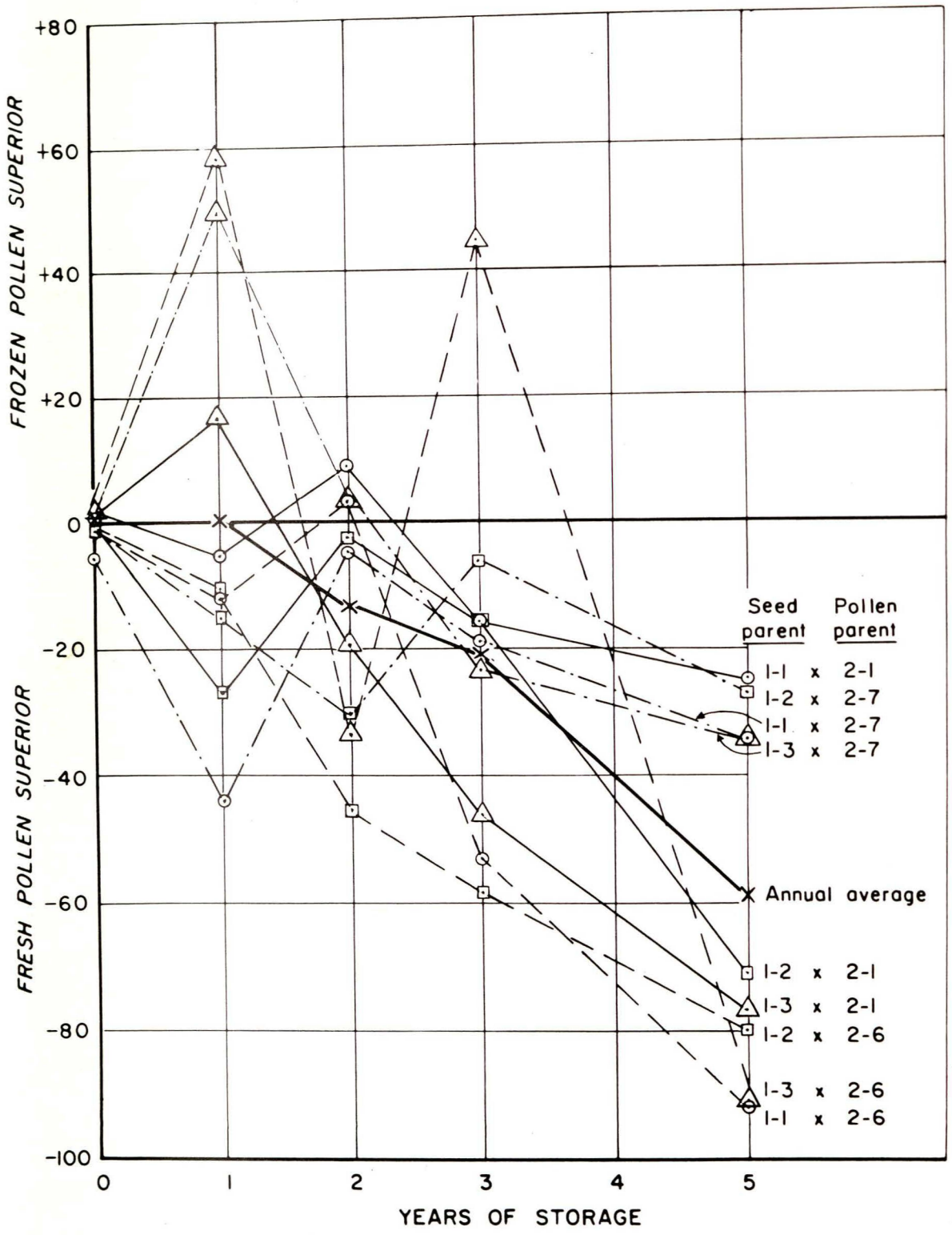


FIGURE 1. — Difference between percent of total seeds that were filled from frozen pollen and from fresh pollen for crosses on *Pinus monticola* during 5 years of pollen storage.

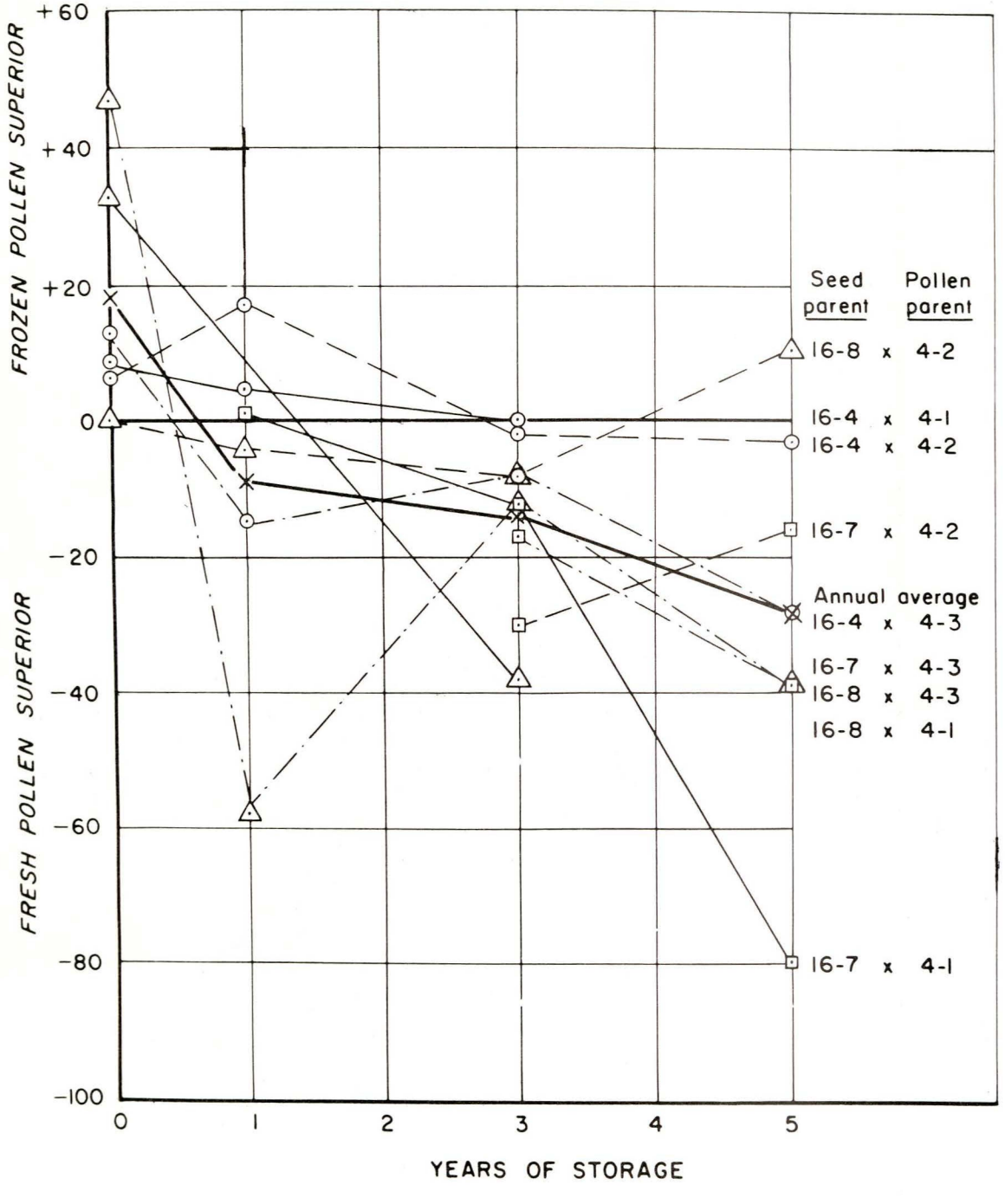


FIGURE 2. — Difference between percent of total seeds that were filled from frozen pollen and from fresh pollen for crosses on *Pinus jeffreyi* during 5 years of pollen storage.

higher yields of filled seeds than did frozen pollen. Frozen pollen also produced more total seed for *P. jeffreyi* and *P. monticola* but not for *P. sabiniana*. The increase was statistically significant only for *P. jeffreyi*. The indication that brief storage of pollen in a freezer increases seed yields requires further study for verification. Brief freezing apparently had a beneficial effect on pollen, but it may not be related to a change in pollen physiology.

Storing pollen in a freezer for 1 year had no significant influence on total seeds per cone or the proportion of the seeds that were filled. Data for three *P. monticola* seed trees showed that frozen pollen had a great advantage over fresh pollen on tree 1 - 3. This unique response of tree 1 - 3 to frozen pollens was not repeated in other years, and it cannot be explained. These results show that pollen frozen for 1 year does not lose its ability to set seed and to produce embryos. Probably all pine breeders should regularly freeze pollen that must be stored for a year before it is used.

The only species producing sufficient strobili for pollination in 1960 was *Pinus monticola*. Thus, data on effects of 2 years of storage are limited. The few cones that were produced on this species gave only 60 percent of normal seed yields. The frozen pollen produced fewer filled seeds per cone than fresh pollen, but the difference was not statistically significant. The proportion of all seeds that were filled following pollination with fresh pollen was about the same as in all other years. Thus, when few strobili are produced in some years, they can be expected to give normal proportions of sound seed but comparatively few sound seeds per cone. Breeding probably should be deferred when strobili are sparse.

Pollinations in 1961 with pollen frozen for 3 years produced significantly fewer seeds than pollinations with fresh pollen. This was true for both *P. monticola* and *P. jeffreyi*. Both total seeds per cone and proportion of seeds that were filled were less with frozen pollen. Crosses on two seed trees of *P. contorta* var. *murrayana* gave results that support these findings. Results of crosses on one seed tree of *P. ponderosa*, however, showed no deleterious effects of freezing pollen for 3 years.

Pollen frozen 5 years lost about half of its ability to produce sound seed. Frozen pollen produced about one-third as many filled seeds as fresh pollen for *P. monticola* and about two-thirds as many for *P. jeffreyi*. Differences between fresh and frozen pollen were statistically significant. Nevertheless, even after 5 years of storage some frozen pollens gave very high yields of filled seeds. These general conclusions are supported by the fragmentary data for the other three species.

On the average, frozen pollens declined gradually but steadily in ability to produce filled seeds after the first year (figs. 1 and 2). The initial increase in production of filled seeds after freezing pollen for a few days has already been mentioned. After 1 year of storage, frozen pollen produced about the same proportion of filled seed as did fresh pollen. The first significant decline occurred after 2 years of freezing. But even after 3 and 5 years of storage the pollens still would be useful in a breeding program. The remaining series of pollinations in this study will show how long frozen pine pollen retains its ability to produce sound seeds.

Pollen frozen 5 years did not lose its ability to set seeds. It kept ovules alive until seedcoats formed. Total number of seed in cones of *P. monticola* remained about the same after 1, 2, and 5 years of freezing, but in the third and fifth years frozen pollen gave fewer seeds per cone than fresh pollen. Crosses of *P. jeffreyi* that set cones did not show a significant reduction in total seeds. These results show that pollens frozen 5 years generally may be expected to set almost normal numbers of cones and seeds.

Pollen from certain trees was better than pollen from other trees after 5 years of freezing. The best pollen parent of *P. monticola* was tree 2 - 7. Pollen from this tree produced the most seeds per cone and the highest proportion of sound seeds. Pollens from trees 2 - 1 and 2 - 6 produced progressively lower proportions of sound seed. For *P. jeffreyi* pollen from tree 4 - 2 fertilized almost the same proportion of seeds after 5 years of freezing as at the beginning of the study. Pollen from tree 4 - 3 retained less of its ability to set sound seed. Pollen from tree 4 - 1, after performing normally in 1961, seemed to have lost nearly all of its viability in 1963. It set cones on two seed trees, but they failed to mature. It produced only one sound seed in five cones on the other seed tree. Perhaps the particular frozen aliquot used in 1963 was defective. Final conclusions on pollen from this tree must await the results of crosses after the next pollinations. Reasons for differences among trees in pollen viability are unknown. Maturity of the pollen when it was collected or its moisture content when placed into storage both might influence storage life. The data show that performance of a pollen after even 3 years of freezing cannot be used to predict its ability to produce seeds after 5 years.

Data for crosses using fresh pollen provide a unique opportunity to analyze compatibilities of seed and pollen parents during five different breeding seasons. The seed data for *P. monticola* were subjected to multiple graphical *t* tests. For each cross the mean plus or minus two standard errors of the mean was plotted (fig. 3). Crosses were considered significantly different if the plotted values did not overlap.

For seed tree 1 - 1, pollen parents did not significantly influence number of filled seeds, total number of seeds, or proportion of filled seeds. The same was true for seed trees 1 - 2 and 1 - 3. Thus, pollen parents did not significantly influence seed production on any of the three seed trees. Seed parents did differ significantly in seed production. Generally the significant differences reflect the low total numbers of seeds set on tree 1 - 2. The

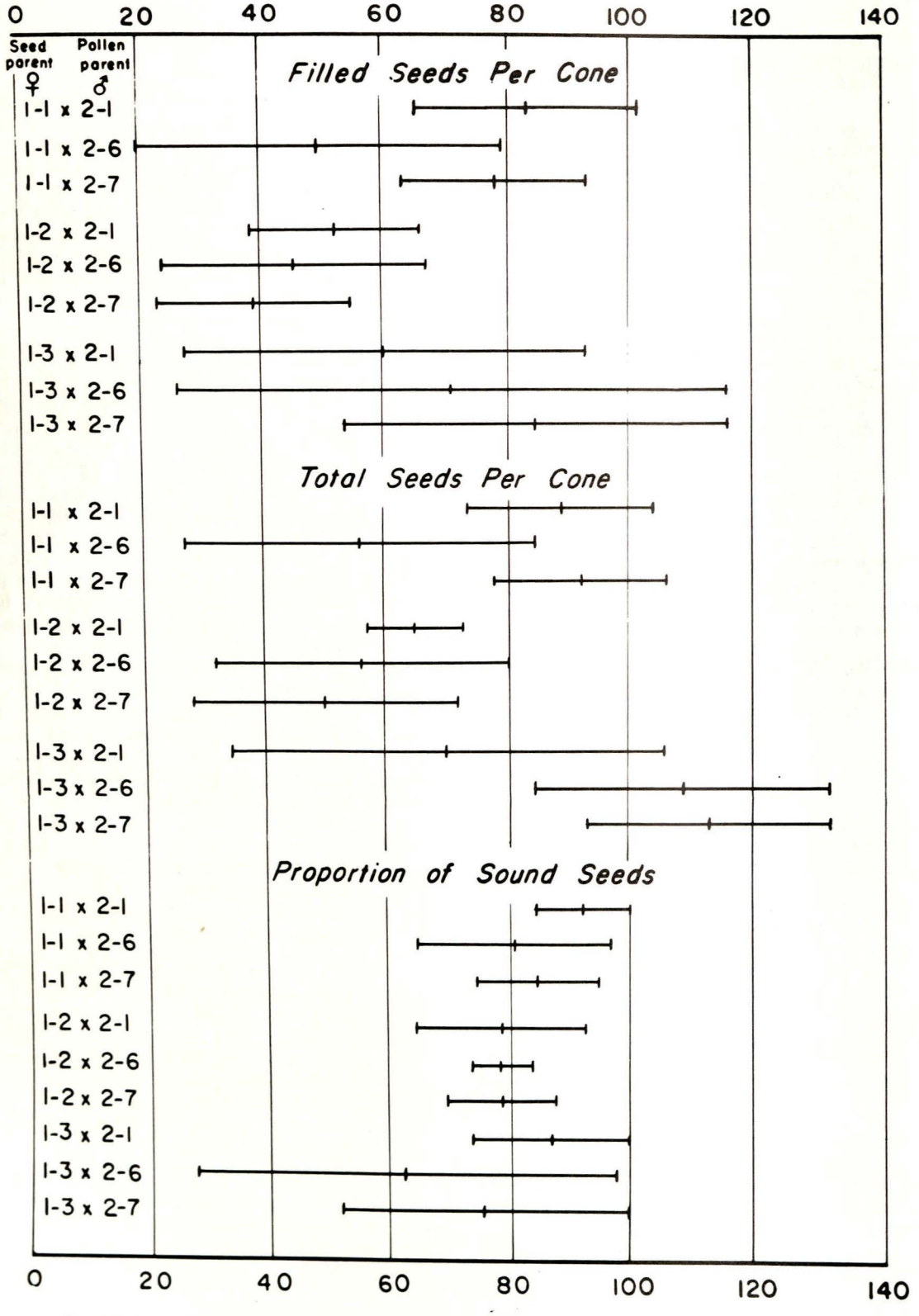


FIGURE 3. — Comparisons of means plus or minus two standard errors of the mean for seed produced by fresh pollen in nine crosses of *Pinus monticola* during 5 breeding seasons.

proportion of seeds that were filled was about the same for all crosses. Seed parents, pollen parents, or particular combinations of seed and pollen parents did not give significant differences. No significant incompatibilities were detected among the nine parental combinations.

Refrigerating pollen at 5°C. during the first 2 years of the test showed no advantages. After 1 year of storage it produced, on the average, less than 10 percent of the number of seeds produced by fresh or frozen pollen. Refrigerated pollen of *P. ponderosa* performed better than that of any other species. It yielded nearly half as many seeds as frozen pollen and one-third as many as fresh pollen. Pollen refrigerated for 2 years could be tested only on *P. monticola* in 1960, and it failed to produce any filled seed. From these results, storing pollen at 5°C. even for 1 year cannot be recommended.

These *in vivo* results show that caution should be used in interpreting the outcome of crossing experiments using year-old refrigerated pollens. Wright (1959) used year-old refrigerated pollen and had results from intraspecific and interspecific crosses similar to ours. Seed production following pollinations with year-old refrigerated pollen cannot be used to estimate genetic incompatibilities between species or seed production potentials from controlled crosses. Neither can seed production by several 1-year-old refrigerated pollens be compared because they differ in rate of deterioration in storage.

This study provided excellent data for comparing seed production from open and controlled pollinations (table 1). Average results from controlled crosses with three fresh pollens were compared with results from open pollination by wind. The

Table 1.--Total seeds and percent of seeds that were filled following controlled pollinations with fresh pollen (C) and open pollinations by wind (O)

| Species | Seed tree | Pollen | Pollination year | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | 1958 | | 1959 | | 1961 | | 1963 | |
| | | | Filled | Total | Filled | Total | Filled | Total | Filled | Total |
| | | | Percent | No. | Percent | No. | Percent | No. | Percent | No. |
| P. monticola | 1-1 | C | 94 | 78 | 75 | 76 | 92 | 82 | 96 | 105 |
| | | O | 76 | 40 | 75 | 193 | -- | -- | -- | -- |
| | 1-2 | C | 87 | 56 | 83 | 44 | 66 | 63 | 87 | 81 |
| | | O | 73 | 33 | 76 | 84 | -- | -- | -- | -- |
| | 1-3 | C | 91 | 130 | 32 | 97 | 73 | 102 | 93 | 105 |
| | | O | 79 | 68 | 94 | 82 | 65 | 83 | -- | -- |
| P. jeffreyi | 16-4 | C | 85 | 130 | 88 | 98 | 96 | 158 | 86 | 132 |
| | | O | 91 | 124 | 94 | 138 | -- | -- | -- | -- |
| | 16-7 | C | -- | -- | 85 | 78 | 96 | 142 | 77 | 119 |
| | | O | -- | -- | 69 | 86 | -- | -- | -- | -- |
| | 16-8 | C | 57 | 109 | 88 | 174 | 92 | 125 | 61 | 136 |
| | | O | 69 | 127 | 73 | 151 | 92 | 72 | -- | -- |
| P. contorta v. murrayana | 13-1 | C | -- | -- | 38 | 32 | 71 | 23 | 83 | 18 |
| | | O | 94 | 11 | 90 | 38 | -- | -- | -- | -- |
| | 13-2 | C | -- | -- | 84 | 17 | 94 | 22 | 92 | 28 |
| | | O | 55 | 15 | 56 | 27 | -- | -- | -- | -- |
| | 13-3 | C | 70 | 8 | 74 | 9 | 89 | 6 | 75 | 23 |
| | | O | 91 | 35 | 91 | 31 | -- | -- | -- | -- |
| P. ponderosa | 131-1 | C | -- | -- | -- | -- | -- | -- | 33 | 74 |
| | | O | -- | -- | -- | -- | -- | -- | -- | -- |
| | 131-3 | C | -- | -- | 72 | 29 | 91 | 54 | -- | -- |
| | | O | -- | -- | 84 | 19 | -- | -- | -- | -- |
| | 131-4 | C | -- | -- | 94 | 75 | 88 | 39 | 75 | 88 |
| | | O | -- | -- | 91 | 90 | 74 | 31 | -- | -- |
| P. sabiniana | 12-1 | C | 64 | 96 | 96 | 86 | -- | -- | 89 | 40 |
| | | O | 50 | 96 | 76 | 46 | -- | -- | -- | -- |
| | 12-2 | C | -- | -- | -- | -- | -- | -- | 81 | 96 |
| | | O | 66 | 141 | -- | -- | -- | -- | -- | -- |
| | 12-3 | C | 70 | 95 | 99 | 31 | -- | -- | 32 | 82 |
| | | O | 13 | 61 | 45 | 111 | -- | -- | -- | -- |

data are extremely variable, but 25 pairs of data were available for 5 species during 4 pollination years. Sign tests (Siegel 1956) showed that controlled and open pollinations did not differ significantly in total seed production or in proportion of seeds that were filled.

Pollen germination *in vitro* is reported here mainly to complete the story. Pollen germination tests were not designed for statistical comparisons of results. Tests were not conducted on the same day or even in the same month in some years, and provisions for replication and randomization in the tests were inadequate.

Fresh pollen collected in successive years had consistently high viability (table 2). After the second and third years of storage the frozen pollens showed germinability equal to that of fresh pollen; their decreasing ability to produce sound seeds has already been discussed. Refrigerated pollens, in agreement with *in vivo* results, showed a marked reduction in germinability after only 1 year of storage and very low germinability after

Table 2.-- Percent of pollen grains germinating or bursting for fresh pollen collected each year and for pollen collected in 1958 and stored in a freezer at -20°C. or in a refrigerator at 5°C.

| Species | Pollen parent | Pollen | Year of germination test | | | | |
|---|---|---|---|---|---|---|---|
| | | | 1958 | 1959 | 1960 | 1961 | 1963 |
| P. monticola | 2-1 | Fresh | -- | 70 | 70 | 41 | -- |
| | | Frozen | -- | 75 | 67 | 45 | 22 |
| | | Refrig. | -- | 60 | 32 | 30 | -- |
| | 2-6 | Fresh | -- | 50 | 50 | 74 | -- |
| | | Frozen | -- | 77 | 77 | 77 | 52 |
| | | Refrig. | -- | 50 | 6 | 4 | -- |
| | 2-7 | Fresh | -- | 80 | 60 | 77 | -- |
| | | Frozen | -- | 37 | 80 | 69 | 57 |
| | | Refrig. | -- | 40 | 4 | 4 | -- |
| P. jeffreyi | 4-1 | Fresh | 60 | 0 | 70 | 75 | 70 |
| | | Frozen | -- | 39 | 65 | 55 | 22 |
| | | Refrig. | -- | 0 | 0 | 0 | -- |
| | 4-2 | Fresh | 70 | 60 | 80 | 85 | 75 |
| | | Frozen | -- | 55 | 70 | 72 | 63 |
| | | Refrig. | -- | 30 | 0 | 10 | -- |
| | 4-3 | Fresh | 90 | 0 | 90 | 49 | 90 |
| | | Frozen | -- | 0 | 60 | 19 | 35 |
| | | Refrig. | -- | 5 | 0 | 0 | -- |
| P. contorta v. murrayana | 12-7 | Fresh | 50 | -- | 90 | 70 | 80 |
| | | Frozen | -- | 54 | 77 | 47 | 38 |
| | | Refrig. | -- | 60 | 5 | 14 | -- |
| | 12-8 | Fresh | 70 | -- | 70 | 67 | 70 |
| | | Frozen | -- | 57 | 80 | 40 | 57 |
| | | Refrig. | -- | 50 | 9 | 9 | -- |
| | 12-9 | Fresh | 60 | -- | 80 | 60 | 78 |
| | | Frozen | -- | 30 | 59 | 34 | 60 |
| | | Refrig. | -- | 60 | 19 | 22 | -- |
| P. ponderosa | 4-59 | Fresh | 80 | 70 | 70 | 26 | 80 |
| | | Frozen | -- | 65 | 87 | 70 | 45 |
| | | Refrig. | -- | -- | 77 | 74 | -- |
| | 4-61 | Fresh | 80 | -- | 60 | 72 | 70 |
| | | Frozen | -- | 69 | 80 | 75 | 50 |
| | | Refrig. | -- | -- | 67 | 60 | -- |
| | 4-65 | Fresh | 90 | 80 | 80 | 80 | 10 |
| | | Frozen | -- | 64 | 90 | 69 | 45 |
| | | Refrig. | -- | -- | 62 | 57 | -- |
| P. sabiniana | 11-2 | Fresh | 80 | 80 | 80 | 70 | 88 |
| | | Frozen | -- | 44 | 64 | 55 | 50 |
| | | Refrig. | -- | 60 | 27 | 17 | -- |
| | 11-3 | Fresh | 80 | 90 | 80 | 80 | 85 |
| | | Frozen | -- | 57 | 62 | 55 | 58 |
| | | Refrig. | -- | 70 | 52 | 18 | -- |

2 and 3 years of storage. The exception to this story was pollen of *P. ponderosa*. Even after 3 years of refrigeration it germinated very well. Unfortunately it was not used in controlled pollinations, so its ability to produce sound seeds is unknown.

Normal cone and seed sets were produced by fresh and frozen pollens of *P. jeffreyi* that failed to germinate *in vitro* in 1959 (fig. 2). Other parallel results of seed production from pollen that failed to germinate *in vitro* are on record at Placerville.

The conclusion to be drawn from all of these findings is that pollen germinability *in vitro* indicates pollen viability, but only use of pollen *in vivo* will show its ability to set cones and to produce sound seeds. Germination tests *in vitro* probably have very limited value in predicting the ability of pine pollens to produce sound seeds.

## Summary

The ability of pollens of five species of pines to set cones, to produce seed, and to yield filled seed after cold storage up to 5 years was determined. Pollen samples for each species were frozen at —20°C. for a few days and for 1, 2, 3, and 5 years. Two other samples for each species were refrigerated at about 5°C. for 1 and 2 years. Each pollen sample was tested for germination *in vitro* in the year in which it was used. Strobili on three seed trees of each species were pollinated with fresh and stored pollen from three other trees. Filled and hollow seeds from each cross were counted. Total seeds per cone and the proportion of seeds that were filled are reported.

Brief freezing of pollen for a few days between extraction and use significantly increased yield of filled seed. Storing pollen in a freezer for 1 year had no significant influence on seed yields. Pollen stored 2 years produced fewer filled seeds than fresh pollen. After 3 and 5 years of freezing, the reductions in yield of sound seeds were significant, becoming progessively greater as storage time increased. Results varied by species, but pollen frozen 5 years produced only about half as many filled seeds as fresh pollen.

However, freezing of pollen did not influence its ability to pollinate strobili and to permit seed coats to form. Brief freezing for a few days actually increased yield of seeds. Total yields of filled plus hollow seeds were about the same for pollen frozen for 5 years as for fresh pollen.

Pollens from different trees varied in ability to produce seeds after 5 years of storage. Pollen from one tree fertilized almost the same proportion of seeds after 5 years of freezing as at the beginning of the study. Another pollen seemed to have lost nearly all of its viability. Reasons for differences among pollens are unknown.

Pollens refrigerated at 5°C. for 1 year produced less than 10 percent of the number of seeds produced by fresh or frozen pollen. Species varied considerably in seed production after 1 year of storage. Pollen refrigerated for 2 years failed to produce any filled seeds.

Successive crosses using fresh pollens in five different breeding seasons failed to show any significant differences in seed setting ability among the pollen parents. Crosses in a year when few strobili were produced resulted in major reductions in seed yields. No genetic incompatibilities were apparent among the nine parental combinations produced each year.

No significant differences were found in seed production following controlled pollinations with fresh pollen and open pollination by wind. Controlled pollination did not result in more or less seed being produced.

Results of pollen germination tests *in vitro* are presented and discussed. The conclusion was drawn that results of such tests have little value in predicting the ability of pine pollens to produce sound seeds.

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# VACUUM STORAGE OF YELLOW-POPLAR POLLEN

by James R. Wilcox[1]

Vacuum-drying, followed by storage *in vacuo* or in an inert gas, is effective for storing pollen of many species. It permits storage at room environments without rigid controls of either temperature or humidity, an advantage that becomes paramount during long-distance transfers of pollen when critical storage conditions are impossible to maintain. In the study reported here, the objectives were to learn if vacuum storage is effective for yellow-poplar (*Liriodendron tulipifera* L.) pollen and to determine optimum rehydration conditions.

## Methods

Yellow-poplar flowers were removed from trees 1 to 2 days prior to anthesis and placed in containers of water. Early in the morning of the day they were expected to shed, they were brought into the laboratory. The gynoecium, sepals, and petals were removed from each flower, and the ring of anthers attached to the stem was placed on a pane of glass. Laboratory temperatures ranged from 68-78° F and humidities from 55-90 percent. By mid-afternoon the anthers had dehisced and the pollen was shaken free by jarring the anthers against the glass. Fifteen to twenty flowers were required to produce 5 milliliters of pollen.

Approximately 5 milliliters of pollen, without pretreatment, were placed in each of fifteen 25-milliliter ampoules that were immediately attached to the ports of a freeze-dryer (King 1961) and dried for 0.5, 2, and 8 hours. Timing started when the vacuum pump had reduced the pressure to 0.05-0.10 mm. of mercury. At the end of each designated time five ampoules were sealed by melting the necks with a torch. All ampoules were stored in the uncontrolled environment of the laboratory. Two check lots of pollen were simply stored in cotton-stoppered bottles, one in the laboratory and one in a refrigerator at 5° C.

The drying process is referred to as auto-freezing by King (1961) and as vacuum-drying by Layne and Hagedorn (1963). Since no prefreezing was done and subfreezing temperatures of samples, as measured by a thermocouple in one ampoule, were not reached, the terminology of Layne and Hagedorn will be used.

The five ampoules representing each drying period were randomly assigned to five storage periods: 0 days (opened immediately), 45 days, and 3, 6, and 12 months. When the ampoules were opened duplicate pollen samples from each were immediately checked for moisture content and germination percent, and additional samples were rehydrated at 25, 50, and 75 percent relative humidity at room temperature and at 5° C for 16 days. Pollen samples were removed from each of the rehydration treatments at 1, 2, 4, 8, and 16 days and checked for germination. Moisture content was based on initial weight and on dry weight following 1 hour's exposure to 100° C. Pollen was germinated on a medium comprised of 1 percent agar and 10 percent sucrose. Germination percentage was computed from a count of 200 grains per sample after 8 hours' incubation in a moist chamber at laboratory temperature. A grain was counted as germinated if the length of the germ tube exceeded the diameter of the grain.

## Results

*Immediate effects of treatment.* — Vacuum-drying decreased the moisture content from 27 to 4.2 percent in the first half-hour, to 2.8 in 2 hours, and to 1.6 percent in 8 hours. This pattern is in general agreement with results reported for birch (Jensen 1964) and pine pollen (Jensen 1964; Ching and Ching 1964).

Germinability declined as drying proceeded. Initial germination of fresh pollen was 83 percent. This decreased to 20, 17, and 7 percent after 0.5, 2, and 8 hours of vacuum-drying. In contrast, King (1959) reported no decrease in viability of loblolly pine pollen immediately following as much as 3 hours of freeze-drying. Jensen (1964) noted a slight decrease in germinability of pine and birch pollen immediately after vacuum treatment, but no decrease in seed set in controlled pollinations.

*Effects of storage.* — Germinability of all samples decreased as storage time increased (table 1). Most striking was the drop from 89 to 0 percent of the untreated check stored at room temperature for 45 days. Subsequent tests have shown that fresh pollen loses all viability after 10 to 14 days' storage at room temperature.

Undried samples stored at 5° C germinated better than the evacuated samples stored at room temperatures. After 12 months, samples stored at 5° C still averaged 7 percent germination.

Comparisons among vacuum-dried samples, following exposure to optimum rehydration conditions, indicated that 0.5 hour of drying resulted in the highest germination through 6 months' storage. Germination at the end of 12 months varied from 1 to 2 percent for all vacuum-dried treatments. The decrease in germination over time was more rapid than what Jensen (1964) reported for pine and birch pollen stored under similar conditions.

[1]*Geneticist at the Institute of Forest Genetics, Southern Forest Experiment Station, Forest Service, U. S. Department of Agriculture, Gulfport, Mississippi.* *The author thanks Dr. J. R. King for introducing him to the vacuum process and reviewing the manuscript.*

Table 1.--Germination of controls and vacuum-dried pollen samples following exposure to optimum recovery conditions (in percent)

| Storage time (months) | Controls stored at: 25° C | Controls stored at: 5° C | Vacuum-dried for: 30 minutes | Vacuum-dried for: 2 hours | Vacuum-dried for: 8 hours |
|---|---|---|---|---|---|
| 0 | 89 | 77.5 | 20.5 | 17.5 | 7.0 |
| 1.5 | 0 | 33.5 | 21.0 | 2.5 | 1.5 |
| 3 | 0 | 33.0 | 8.5 | 2.5 | 2.5 |
| 6 | 0 | 19.5 | 6.5 | 3.0 | 2.0 |
| 12 | 0 | 7.0 | .5 | 1.0 | .5 |

*Effects of rehydration.* — King (1961) has discussed the importance of environment in the rehydration of vacuum-dried samples. He reported that loblolly pine pollen exposed for 48 hours to 60 percent relative humidity at 5° C germinated four times as well as that cultured immediately after opening.

In the present study rehydration increased germination in varying degrees. Results for samples vacuum-dried for 30 minutes are presented in figure 1. At equal relative humidities, samples rehydrated at 5° C consistently germinated better than those rehydrated at room temperature. Within the two temperature regimes, relative humidity affected the germination percent, particularly at 5° C. Samples exposed for 24 hours to 75 percent relative humidity at 5° C germinated three times as well as non-rehydrated samples. Changes in germination percent after 24 hours appear to be a reflection of storage conditions and demonstrate the importance of using the pollen as soon as possible after rehydration. The samples vacuum-dried for 2 and 8 hours and stored for the various times responded in the same way to the different rehydration conditions.

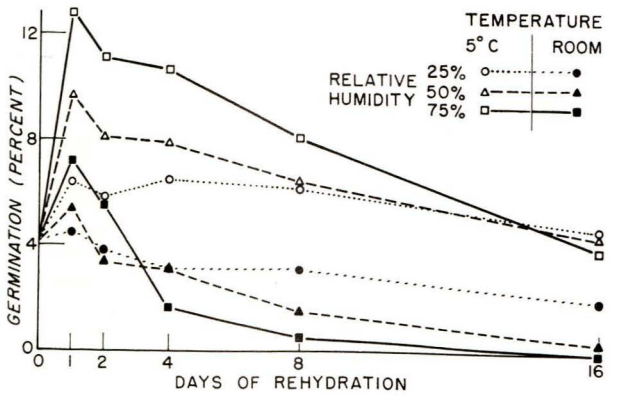


FIGURE 1. — Germination response, following exposure to various rehydration conditions, of pollen vacuum-dried for 30 minutes.

## Discussion and Conclusions

While vacuum-drying yellow-poplar pollen permitted storage at room temperature without the rapid and complete loss of viability suffered by undried samples, it nevertheless reduced germinability considerably. Reducing evacuation time or storing dried samples at low temperatures, or both, may alleviate this difficulty and permit shipment of pollen over great distances. Such shipment has been successful for other genera (King 1961, 1965).

Germination of samples dried for 30 minutes was 8.5 percent after 3 months' storage. Although this is a drastic reduction from germinability of fresh pollen, studies with other species (Callaham and Duffield 1961) indicate that it may still be adequate for good seed set from controlled breeding. Pollinations have been made with samples from all the treatments, with checks stored for 1 year, and with fresh pollen.

The relatively high germinability of undried pollen stored at 5° C indicates this is the best storage method of those tested where long-distance transfers are not anticipated.

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# FIELD TRIP ON THE KELLOGG FOREST OF MICHIGAN STATE UNIVERSITY NEAR AUGUSTA, MICHIGAN

**Tour Guides: Jonathan W. Wright and Walter Lemmien[1]**

When the Kellogg Forest was established in 1932, 90 percent of the farmland in the locality was idle. The original tree cover had been oak-hickory on the upland areas and basswood, white ash, American elm, and black cherry on the lower, more fertile areas. Agricultural activities had begun between 1830 and 1850. Since then, continued cropping had resulted in severe erosion and eventual abandonment of most of the farms.

The first objective of the Forest was to demonstrate reforestation practices for erosion control and production of usable crops. Since 1948, however, the primary function of the Forest has been research. Much of the area now is devoted to forest tree improvement research plots. Those plots visited by members of the Conference are described in the following pages.[2]

## Placerville Hybrid Pines (CPT. 25* D)

In the late 1940's the Institute of Forest Genetics at Placerville, California, artificially produced pine hybrids of several combinations.

Seeds of some of these hybrids were received and sown in the Michigan State University Nursery in the spring of 1950. In April 1953, the 3-0 stock was hand-planted in furrows on a sandy loam with a heavy sod cover. Two local seedlots were included as controls.

The following hybrids have been tested:

*P. ponderosa* X *P. arizonica* (mostly dead now from repeated winter injury).

*P. ponderosa* X *P. apacheca* (exhibit a moderate amount of damage).

*P. ponderosa* X *P. ponderosa* var. *scopulorum* (growing very well).

*P. contorta* X *P. banksiana* (show no hybrid vigor but superior form).

*P. monticola* (California origin) X *P. strobus* (mostly dead).

*P. monticola* (Idaho origin) X *P. strobus* (excellent growth the last 2 years).

## Scotch Pine Provenance Tests (MSFGP 2/3/4 – 61, MSFGP 3/4 – 63)

The first 121 seedlots were sown in the nursery in the spring of 1959 and field planted in 1961. The next 68 seedlots were sown in 1961 and field planted in 1963. Much of the material was included in NC-51 tests and therefore is represented in a number of States.

Scotch pine has a very large natural range, stretching from Spain and Scotland in the West to Turkey in the Southeast and Eastern Siberia in the East. In the Northern and Eastern parts, the range is continuous. In the Western and Southern parts the range is discontinuous; it comprises a number of isolated stands on mountains. Based on the test plantings, results to date are about as follows:

1. There are distinct source-related differences in a number of characteristics. In height, needle color, and needle length a very small proportion of the total variance may be due to interaction between seedlot and plantation.

2. The genetic variation pattern for the species appears to be discontinuous.

3. The average 6-year (from seed) heights of the different varieties show that the tallest is variety *haguenensis* from the Vosges Mountains of France and adjacent Germany.

4. There is a general relationship between height and sawfly attack.

5. Growth-chamber experiments show that the foliage change from winter yellow to summer green takes place in light if the daytime temperature reaches 45°F.

## European Black Pine Provenance Test (MSFGP-5 - 61)

European black pine is the most widespread pine species of southern Europe. Its natural range extends from Spain to the Crimea and Turkey. Generally it grows on calcareous soils and to the south of, or at lower elevations than, Scotch pine. The usual common name is Austrian pine.

Twenty-seven seedlots were received from Greece, Spain, Corsica, France, Yugoslavia, Austria, Turkey, and the Crimea. The seedlings were field planted as 2-0 stock in the spring of 1961. Half the trees in each plot at Kellogg were given a nitrogen fertilizer in the spring of 1964.

Results so far seem to indicate six varieties within the species: (1) Austria and Yugoslavia, (2) Eastern Mediterranean, (3) Southern Italy, (4) Corsica, (5) French mainland, and (6) Spain.

The Corsican sources have produced trees very different from the rest and generally of rather poor hardiness. The Spanish seedlots are also rather distinct from the balance of the sources. The commonly used Austrian source is among the slowest

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[2] *Further details about plots visited can be obtained from Jonathan Wright.*

growing of all the hardy seedlots. The fertilizer trials show no significant fertilizer-genotype interactions.

### White Pine Provenance Test (MSFGP-3-60)

In April 1959, the Northeastern Forest Experiment Station shipped 2-0 seedlings of 15 seedlots of eastern white pine to Michigan State University. This material is part of the rangewide provenance study started by the U.S. Forest Service in 1955.

The stock was lined out for a year in the nursery at East Lansing and field planted in April 1960.

Results of the field planting through age 6 indicate that (1) the Georgia and Tennessee seedlots are the fastest growing; (2) flowering first started in 1965; (3) there are significant differences in time of leaf fall as between sources; (4) mortality has been fairly low, but has been greatest for the very slow-growing Minnesota and Nova Scotia origins and the very fast-growing southern Appalachian origins.

### Virginia Pine Provenance Study (MSFGP-1-60)

Twenty seedlots of Virginia pine were field planted in April 1960 as 1-1 stock. Results to date have been as follows:

1. Most of the mortality has occurred in the southern origins (Mississippi, Alabama, Tennessee, western Kentucky, and western North Carolina).
2. Winter burn is noticeable on the needles of southern origins every spring.
3. There are significant between-origin differences in average height at age 8. The slowest growing sources are southern but the pattern is not clear.
4. Flowering started in 1962 at age 5 from seed and has been light on three southern seedlots.
5. There appear to be no significant differences among seedlots in mycorrhizal developments.

### Ponderosa Pine Provenance Test

In the fall of 1959, seed was obtained from the Institute of Forest Genetics at Placerville, California, from 300 single trees in 60 different stands over the ponderosa pine range. Stock was grown and field planted in April 1962.

Results so far indicate a number of distinct ecotypes within the species as follows: (1) California-Nevada, (2) coastal Oregon, (3) interior parts of the Pacific Northwest, (4) Arizona, southern New Mexico, (5) Utah, and (6) eastern Montana, Black Hills, and Nebraska.

### Limber-Border Pine Provenance Test (MSFGP-11-64)

The limber-border pine work was started in 1959 by J. W. Andresen under a National Science Foundation grant. During 1959 and 1960 open-pollinated seeds were collected from 500 individual trees and about 60 stands scattered throughout the Rocky Mountain Region. Stock grown from these seeds was field planted in the spring of 1964 as 2-1 transplants.

Field planting results indicated a clear separation between a fast-growing, long-needled, many-cotyledon, blue-green, slightly serrated type from Arizona-New Mexico and the slower growing types to the north. The northern population has been called *Pinus flexilis* James and the southern one, border pine, *P. strobiformis* Engelmann. Within either species there is some evidence of races. The *P. strobiformis* has survived considerably better and grows faster than the *P. flexilis*.

### Red Pine Provenance Test (MSFGP-2-63)

Ninety-three seedlots covering most parts of the red pine range were sowed in the nursery at East Lansing and field planted as 3-0 stock in 1963.

No results from the field planting are yet available, but the 2- and 3-year data in the nursery showed greater height growth for provenances from southern Michigan and central Wisconsin than for other origins.

### Austrian-Japanese Red Pine Hybrids

The natural hybrids occur on the north and west sides of a 30-year-old *Pinus nigra* stand. They apparently resulted from natural pollination by a *P. densiflora* stand of similar age about 300 feet to the west.

The growth rate of the older hybrids (7 or more years old) is very promising. The tallest tree is 18 feet and growing nearly 3 feet per year. The second tallest grew 63 inches in the past 2 years. The form seems very good; better than that of either parent.

### Himalayan White Pine

In 1959 it was noted that 13 trees in 40 acres of eastern white pine plantations were Himalayan white pine (*Pinus griffithii* McClel.). Compared to the eastern white pines growing closest to them, the Himalayan white pines are slightly shorter, slightly larger in diameter, and definitely more heavily weevilled (difference significant). Interest in the Himalayan white pine centers around its (1) known greater resistance to blister rust, (2) hybridization possibilities with *P. strobus*, and (3) hybridization possibilities with *P. flexilis*.

### Japanese Larch Provenance Test (MSFGP-2-60, 1-61)

Japanese larch is native to a relatively small area in the mountains of central Honshu. It grows at 4,000 to 7,000 feet elevation in scattered stands within a 140-mile-square area.

Seven seedlots were received in 1957 and 22 in 1958. These were field planted in 1960 and 1961,

respectively. Results so far indicate, (1) interesting and contradictory facts about site preference, (2) fairly consistent genetic uniformity, (3) consistently less winter dieback and earlier leaf fall in seedlots from the northeastern part of the native range, (4) statistically significant variation of growth rates among origins, and (5) strong interaction with environment as to flowering.

### White Spruce Provenance Tests, Ontario and Rangewide (MSFGP-1-60, 5.6.7-63)

Stock from an Ontario-wide provenance study was planted as 2-2-2 in April 1960. 1965 height measurements subjected to analysis of variance showed that differences were significant at the 2-percent level. Cones appeared in 1962 and their numbers have increased since, but between-origin differences have not been significant.

Stock from the entire range of white spruce was field planted as 2-3 transplants in April 1963. The source from near Petawawa, Ontario, has been the fastest growing but is not significantly better than several others. Trees from Alaska, Labrador, and western Provinces of Canada are smaller than average. Slight color differences between origins are statistically significant.

### Douglas-Fir Provenance Test (MSFGP-16-35)

The trials include one set of 142 seedlots and a second set of 60 seedlots from the entire United States range and part of the Canadian range. Results indicate one or more different ecotypes in each of the following areas: (1) coastal section of Oregon, Washington, and British Columbia (generally not very hardy), (2) inland sections, with the fastest growing types from eastern Washington, northern Idaho, and northwestern Montana, (3) Colorado and northern New Mexico, and (4) Arizona and southern New Mexico areas (the fastest growing types are on these areas). The second outplanting also indicated more favorable growth for a source from New Mexico.

### White Fir Provenance Test

Stock of several *Abies concolor* seed origins was planted in 1963. Because of heavy mortality, it was replanted in 1965 with 1-2 stock of 12 origins from Utah, New Mexico, Colorado, and Arizona.

### Grand Fir Provenance Study

Five lots of *Abies grandis* from Montana, northern Idaho, and eastern Washington were planted in 1963, but all origins have suffered severe winter-foliage burn in the nursery and results have not been too promising. There were evidences of a considerable range in height growth within the Rocky Mountain population.

### English Oak—White Oak Plantation (MSFGP-2-63)

*Quercus robur* grown from acorns collected on the Michigan State University Campus and *Q. alba* from the Russ Forest were planted in pairs. From the start, the English oak trees have outgrown the native white oak.

### Red Oak Provenance Test (MSFGP-2-62)

As a part of the rangewide red oak provenance test organized by Howard Kriebel of the Ohio Agricultural Research and Development Center, 16 sources of 2-0 stock were planted in 1962 and 5 more sources in 1963. No valuable genetic data are yet forthcoming, partly as a result of heavy rabbit, and some deer, damage.

# SCIENTIFIC AND COMMON NAMES OF TREE GENERA AND SPECIES MENTIONED IN THE TEXT[1]

| *Scientific name* | *Common name* |
|---|---|
| *Abies concolor* (Gord. & Lindl. | White fir |
| *A. grandis* (Dougl.) Lindl. | Grand fir |
| *Betula alleghaniensis* Britton | Yellow birch |
| *B. glandulosa* Michx. | Bog birch |
| *B. lenta* L. | Sweet birch |
| *B. nigra* L. | River birch |
| *B. papyrifera* Marsh. | Paper birch |
| *B. pendula* Roth. | European white birch |
| *B. pubescens* Ehrh. | (European birch) |
| *B. pumila* L. | Low birch |
| *Larix decidua* Mill. | European larch |
| *L. laricina* (DuRoi) K. Koch | Tamarack |
| *L. leptolepsis* (Sieb & Zucc.) Gord. | Japanese larch |
| *Liriodendron tulipifera* L. | Yellow-poplar |
| *Picea abies* (L.) Karst. | Norway spruce |
| *P. glauca* (Moench) Voss | White spruce |
| *P. glehni* (Fr. Schmidt) Mast. | Sakhalin spruce |
| *P. jezoensis* (Sieb. & Zucc.) Carr. | Yeddo spruce |
| *P. koyamai* Shiras. | Koyama spruce |
| *P. mariana* (Mill.) B.S.P. | Black spruce |
| *P. obovata* Ledeb. | Siberian spruce |
| *P. omorika* (Pancic) Purkyne | Serbian spruce |
| *P. schrenkiana* Fisch. & Mey. | Schrenk spruce |
| *P. sitchensis* (Bong.) Carr. | Sitka spruce |
| *P. spinulosa* (Griff.) Henry | Sikkim spruce |
| *Pinus aristata* Engelm. | Bristlecone pine |
| *P. attenuata* Lemm. | Knobcone pine |
| *P. banksiana* Lamb. | Jack pine |
| *P. contorta* Dougl. (*P. contorta* var. *murrayana* (Grev. & Balf.) Engelm.) | Lodgepole pine |
| *P. coulteri* D. Don | Coulter pine |
| *P. densiflora* Sieb. & Zucc. | Japanese red pine |
| *P. echinata* Mill. | Shortleaf pine |
| *P. durangensis* Martinez | Durango pine |
| *P. elliottii* Engelm. | Slash pine |
| *P. elliottii* var. *densa* Little & Dorman | South Florida slash pine |
| *P. engelmannii* Carr. (*P. apacheca* Lemm.) | Apache pine |
| *P. flexilis* James | Limber pine |
| *P. griffithii* McClelland | Himalayan pine |
| *P. jeffreyi* Grev. & Balf. | Jeffrey pine |
| *P. lambertiana* Dougl. | Sugar pine |
| *P. montezumae* Lamb. | Montezuma pine |
| *P. monticola* Dougl. | Western white pine |
| *P. muricata* D. Don | Bishop pine |
| *P. nigra* Arnold | Austrian pine (European black pine) |
| *P. nigra* var. *caramanica* (Loud.) Rehder | Crimean pine |
| (*P. nigra* var. *calabrica*) | (Calabrian pine) |
| *P. nigra* var. *cebennensis* (Gren. & Godr.) Rehd. | Cevennes pine |
| *P. nigra* var *poiretiana* (Ant.) Aschers. & Graebn. | Corsican pine |
| *P. ponderosa* Laws. | Ponderosa pine |
| *P. ponderosa* var. *arizonica* (Engelm.) Shaw | Arizona pine |
| *P. ponderosa* var. *scopulorum* Engelm. (*P. scopulorum* (Engelm.) Lemm.) | Rocky Mountain ponderosa pine |
| *P. pseudostrobus* Lindl. (*P. oaxacana* Mirov) | Nicaragua pine |
| *P. radiata* D. Don | Monterey pine |
| *P. resinosa* Ait. | Red pine |
| *P. sabiniana* Dougl. | Digger pine |
| *P. strobus* L. | Eastern white pine |
| *P. strobiformis* Engelm. | (Border limber pine) |
| *P. sylvestris* L. | Scotch (Scots) pine |
| *P. taeda* L. | Loblolly pine |
| *P. torreyana* Parry | Torrey pine |
| *P. virginiana* Mill. | Virginia pine |
| *P. washoensis* Mason & Stockwell | Washoe pine |

[1]*Authorities for nomenclature are: Check List of Native and Naturalized Trees of the United States, by Elbert Little, U. S. Dep. Agr. Handbook 41, 1953; Standardized Plant Names, by Harlan P. Kelsey and William A. Dayton, 1942, Harrisburg, Penn.; Manual of Cultivated Trees and Shrubs, by Alfred Rehder, 1940, New York; and Geographic Distribution of the Pines of the World, by William B. Critchfield and Elbert L. Little, Jr., U. S. Dep. Agr. Misc. Publ. 991, 1966.*

| *Scientific name* | *Common name* |
|---|---|
| *Populus alba* L. | White poplar |
| *P. canescens* (Ait.) Sm. | Gray poplar |
| *P. deltoides* Bartr. | Eastern cottonwood |
| *P. grandidentata* Michx. | Bigtooth aspen |
| *P. lasiocarpa* Oliv. | Chinese poplar |
| *P. nigra* L. | Black poplar |
| *P. tremula* L. | European aspen |
| *P. tremuloides* Michx. | Quaking aspen |
| *Pseudotsuga menziesii* (Mirb.) Franco | Douglas-fir |
| *P. menziesii* var. *glauca* (Beissn.) Franco | (Rocky Mountain Douglas-fir) |
| *Quercus alba* L. | White oak |
| *Q. robur* L. | English oak |
| *Q. rubra* L. | Northern red oak |

## LETTER OF APPRECIATION

The following letter, signed by Hans Nienstaedt as Chairman of the SAF Committee on Forest Tree Improvement and Scott Pauley as Chairman of the Lake States Forest Tree Improvement Committee was sent to Dean Richard U. Byerrum, College of Natural Science, Michigan State University, on October 28, 1965:

> On behalf of the Committee on Forest Tree Improvement of the Society of American Foresters and the Lake States Forest Tree Improvement Committee, we should like to express to you our deep appreciation for the fine meeting facilities and services provided us at the W. K. Kellogg Biological Station on October 21 to 23, 1965. During these dates we conducted a Society of American Foresters' Genetics Workshop in conjunction with the Seventh Lake States Forest Tree Improvement Conference. In attendance were about 80 research workers in forest genetics and related fields from all sections of the United States and Canada. The program consisted of 22 technical papers and a one-day field trip to visit the experimental forest plantings at the Kellogg Forest.
>
> We should like to make special mention of the fine treatment accorded us by Mr. Paul Hartman and his efficient staff at the Biological Station and particularly of the fine work done by Prof. Jonathan Wright of the Forestry Department, College of Agriculture, in making arrangements for the Workshop-Conference and for conducting the field trip.
>
> We should appreciate your extending our thanks to those members of your staff who helped make this Workshop-Conference the genuine success that it was.

## BUSINESS MEETINGS

### SAF Tree Improvement Committee Affairs

The SAF Committee on Forest Tree Improvement met on the evening of October 21. The group was informed that the Lake States Forest Experiment Station (now North Central Forest Experiment Station) had agreed to publish the Proceedings.

It was also agreed that the Committee would proceed with the development of a new directory of workers in forest genetics. The two previous directories (for foreign workers and for U.S. workers) will be combined in the revision.

The Committee discussed the desirability of developing reports that would summarize briefly all that is known about the genetic characteristics of important forest tree species. It was agreed that such reports should be limited only to those species for which there was a considerable body of information, and that all sources of information, both published and unpublished, would be tapped insofar as possible. It was not decided whether the Committee would attempt this as a publication project or would act only as the stimulating group and ask the U.S. Forest Service to publish these reports as it did those on silvical characteristics of important tree species of the United States.

As a subgroup, those at the conference especially interested in population genetics conducted seminars on this topic under the direction of Gene Namkoong of the Southeastern Forest Experiment Station and William Libby of the University of California.

Nominations were made for new members to the Committee to replace those whose terms would expire at the end of 1965. These will be transmitted to the Chairman of the Division of Silviculture, Society of American Foresters.

### Lake States Forest Tree Improvement Committee Affairs

**by Paul O. Rudolf**

On the evening of October 21, 1965, the Lake States Forest Tree Improvement Committee met at the W. K. Kellogg Biological Station, Hickory Corners, Michigan, in conjunction with the Society of

American Foresters Committee on Forest Tree Improvement. Members present were Scott Pauley (Chairman), Jonathan Wright (Vice Chairman), Paul O. Rudolf (Executive Secretary), Burton Barnes, W. H. Brener, Dean Einspahr, F. J. Hodge, Gene Meyer, and Donald Lester (for R. F. Patton). Present at the Conference, but not at the meeting were Max Pillow (for H. L. Mitchell) and J. A. Pitcher (for E. N. Lee).

The meeting briefly covered the following four items: Subcommittee activities; new officers; new members; and the next newsletter (Trebredinews).

*Subcommittee reports.* — W. H. Brener, Chairman of the Seed Certification Subcommittee, reported no direct activities by his group. The Executive Secretary did report that an amendment to the Minnesota seed law, to extend coverage to seeds of woody plants, had failed to pass because of delaying tactics (not directed at the tree seed provisions) in the Senate.

Paul O. Rudolf, Chairman of the RECAP (Research Evaluation, Coordination, and Planning) Subcommittee reported that the new survey of forest tree improvement activities in the Lake States was now in rough-draft stage and made comparisons with the results of the 1959 survey.

*Election of officers.* — Results of the balloting by mail gave the following slate of officers for 1966-67:

*Chairman* — Jonathan W. Wright, Michigan State University

*Vice Chairman* — Dean Einspahr, Institute of Paper Chemistry

A change of the representative for the Lake States Forest Experiment Station brings in Hans Nienstaedt as Executive Secretary.

*New members to serve 1966-1969.* — Members of the Lake States Forest Tree Improvement Committee serve 4-year overlapping terms. Consequently half the 14 members are replaced or reappointed every 2 years at the time of the biennial conferences. Replies received by Chairman Pauley from the heads of member organizations indicate that the following will be members of the Committee from January 1, 1966, through December 31, 1969:

W. H. Brener, Wisconsin Conservation Department

Gene Meyer, Lake States Council of Industrial Foresters

Hans Nienstaedt, Lake States Forest Experiment Station

Scott Pauley, University of Minnesota

Jonathan W. Wright, Michigan State University

In addition, the Regional Forester, U.S. Forest Service, North Central (now Eastern) Region, has appointed John A. Pitcher to replace E. N. Lee. His term will extend through 1967.

*Next newsletter.* — The next issue of the Lake States Trebredinews will be due this fall or winter. The various member organizations take turns in assembling, processing, and distributing the newsletter. Jonathan Wright accepted this responsibility for Michigan State University. This completes the rotation; all member organizations will now have put out at least one issue of Trebredinews.

*Next meeting.* — The Lake States Forest Tree Improvement Committee tentatively agreed to meet in the summer or early fall of 1966 to discuss preliminary plans for the next biennial conference. In the normal rotation this conference will be held in Wisconsin in 1967, and Dean Einspahr will act as Program Chairman.

## OFFICERS AND MEMBERS OF THE LAKE STATES FOREST TREE IMPROVEMENT COMMITTEE, 1965

| *Name and address* | *Appointed through** |
|---|---|
| Scott S. Pauley (Chairman)<br>School of Forestry<br>University of Minnesota<br>St. Paul 1, Minnesota | 1967 |
| Jonathan W. Wright (Vice Chairman)<br>Department of Forestry<br>Michigan State University<br>East Lansing, Michigan | 1965 |
| Paul O. Rudolf (Executive Secretary)<br>Lake States Forest Experiment Station<br>St. Paul Campus, University of Minnesota<br>St. Paul 1, Minnesota | 1965 |
| W. H. Brener<br>Griffith State Nursery<br>Wisconsin Rapids, Wisconsin | 1965 |
| Dean Einspahr<br>Institute of Paper Chemistry<br>Appleton, Wisconsin | 1967 |
| Gene Hesterberg<br>Department of Forestry<br>Michigan Technological University<br>Houghton, Michigan | 1967 |
| F. J. Hodge<br>Forestry Division<br>Michigan Conservation Department<br>Lansing, Michigan | 1967 |
| Fred Knight<br>School of Natural Resources<br>University of Michigan<br>Ann Arbor, Michigan | 1967 |
| Emil G. Kukachka<br>Division of Forestry<br>Minnesota Conservation Department<br>St. Paul 1, Minnesota | 1965 |
| E. N. Lee<br>U.S. Forest Service<br>710 North Sixth Street<br>Milwaukee 3, Wisconsin | 1967 |
| Gene Meyer<br>Marathon, A Division of American Can Company<br>Box 481, Ironwood, Michigan | 1965 |
| H. L. Mitchell<br>Forest Products Laboratory<br>Madison 5, Wisconsin | 1967 |
| R. F. Patton<br>Department of Plant Pathology<br>University of Wisconsin<br>Madison 6, Wisconsin | 1965 |
| Burton V. Barnes<br>School of Natural Resources<br>University of Michigan<br>Ann Arbor, Michigan | 1965 |

**Committee tenure; terms of officers expire as of December 31, 1965.*

## THE COMMITTEE ON FOREST TREE IMPROVEMENT, DIVISION OF SILVICULTURE, SOCIETY OF AMERICAN FORESTERS, 1965

Hans Nienstaedt, Chairman, Institute of Forest Genetics, Lake States Forest Experiment Station, Rhinelander, Wisconsin 54501

Robert M. Echols, Institute of Forest Genetics, Pacific Southwest Forest and Range Experiment Station, Placerville, California 95667

C. C. Heimburger, Southern Research Station, Ontario Department of Lands and Forests, Maple, Ontario

Helge Irgens-Moller, School of Forestry, Oregon State University, Corvallis, Oregon 97331

G. A. Limstrom, Central States Forest Experiment Station, 111 Old Federal Building, Columbus, Ohio 43215

Thomas O. Perry, School of Forestry, North Carolina State University, Raleigh, N. C. 27607

F. S. Santamour, Morris Arboretum, University of Pennsylvania, Philadelphia, Pennsylvania 19119

J. C. van Buijtenen, Forest Genetics Laboratory, Texas Forest Service, College Station, Texas 77843

North Central Forest Experiment Station.
1966. Joint proceedings, Second Genetics Workshop of the Society of American Foresters and the Seventh Lake States Forest Tree Improvement Conference, October 21-23, 1965. N. Cent. Forest Exp. Sta., St. Paul, Minn. 110 pp., illus. (U.S. Forest Serv. Res. Paper NC-6.)

Included are (1) 22 technical papers (by researchers from many sections of the United States and Canada) pertaining to selection and progeny testing, radiation genetics, intraspecific variation, natural and artificial hybridization, breeding systems, breeding methodology and specialized tree breeding techniques, and applied breeding and allied fields; (2) concise descriptions of 16 genetic studies visited on the Kellogg Forest of Michigan State University; and (3) brief reports of the activities of the Lake States Forest Tree Improvement Committee and the Society of American Foresters' Committee on Forest Tree Improvement. The meeting was held at Hickory Corners, Mich.

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## PUBLICATIONS ISSUED FOR THE LAKE STATES FOREST TREE IMPROVEMENT COMMITTEE

Proceedings, Lake States Forest Genetics Conference, March 30 - April 1, 1953. Lake States Forest Exp. Sta. Misc. Rep. 22, 83 pp. 1953.

Proceedings, Lake States Forest Tree Improvement Conference, August 30-31, 1955. Lake States Forest Exp. Sta. Misc. Rep. 40, 108 pp., illus. 1955.

Forest Genetics in the Lake States, an Annotated Bibliography, by William J. Libby, Burton V. Barnes, and Stephen H. Spurr. Univ. Mich. School Natur. Resources (no series), 74 pp. 1956.

Guide for Selecting Superior Trees and Stands in the Lake States, by Paul O. Rudolf. Lake States Forest Exp. Sta., Sta. Pap. 40, 32 pp., illus. 1956.

Forest Tree Seed Collection Zones for the Lake States. Mich. Dep. Conserv., Forest. Div. (no series), 13 pp., illus. 1957.

Proceedings, Third Lake States Forest Tree Improvement Conference, Sept. 17-18, 1957. Lake States Forest Exp. Sta., Sta. Pap. 58, 87 pp., illus. 1958.

Forest Tree Improvement Research in the Lake States; A Survey by the Lake States Forest Tree Improvement Committee. Lake States Forest Exp. Sta., Sta. Pap. 74, 56 pp. 1959.

Registering and Marking Selections in the Lake States; A Report of a Subcommittee of the Lake States Forest Tree Improvement Committee, by Paul O. Rudolf and H. E. Ochsner. U.S. Forest Serv. N. Cent. Reg. (no series), 9 pp. 1959.

Forest Tree Seed Certification in the United States; A Report of a Subcommittee of the Lake States Forest Tree Improvement Committee, by J. W. Macon. Consolidated Water Power and Paper Co. (no series), 3 pp. 1959.

Proceedings of the Fourth Lake States Forest Tree Improvement Conference, Oct. 6-7, 1959. Lake States Forest Exp. Sta., Sta. Pap. 81, 60 pp., illus. 1960.

Proceedings of the Fifth Lake States Forest Tree Improvement Conference, Sept. 19-20, 1961. Lake States Forest Exp. Sta., Sta. Pap. 98, 42 pp. 1962.

Report of the Seed Certification Subcommittee of the Lake States Forest Tree Improvement Committee, by W. H. Brener. Wis. Conserv. Dep. (no series), 4 pp., 1963.

Proceedings of the Sixth Lake States Forest Tree Improvement Conference. Sept. 9-10, 1963. Lake States Forest Exp. Sta. (no series), 90 pp. 1964.

## THE FOREST SERVICE CREED

*The Forest Service of the U.S. Department of Agriculture is dedicated to the principle of multiple use management of the Nation's forest resources for sustained yields of wood, water, forage, wildlife, and recreation. Through forestry research, cooperation with the States and private forest owners, and management of the National Forests and National Grasslands, it strives — as directed by Congress — to provide increasingly greater service to a growing Nation.*